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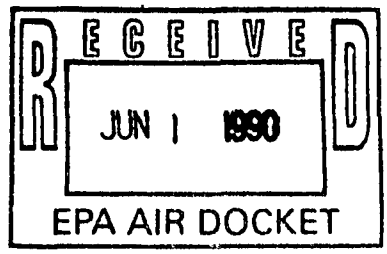
Everett L. Hodges

May 21, 1990



Environmental Protection Agency
RE: Public Docket A-90-16, AIR Docket "LE131"
Room M-1500
Washington, DC 20460

Gentlemen:

I am advised that a Hearing is scheduled for June 22 at which time your agency will consider approving Ethyl Corporation's proposal to add MMT compounds to gasoline.

I ask you to be cautious in adding any manganese based compounds, especially MMT, to the environment until there is a resolution with regards to the findings of the enclosed research. The Department of Psychiatry and Pharmacology, University of California - Irvine, over the last five years have repeatedly verified excessive manganese in tissue of violent individuals from four separate studies in California, versus controls. The enclosed paper, prepared by Dr. Louis Gottschalk, Chairman of the Department., has been submitted to the Journal of Neurotoxicology for publication.

In addition, please find a paper by Donaldson and Barbeau "Manganese Neurotoxicity: Possible Clues to the Etiology cf Human Brain Disorders". This paper deals with the neuro-degenerative effects of manganese and is the result of research Dr. Donaldson and others have conducted over the years. In addition, please find a copy of the National Research Council of Canada's document on manganese in the Canadian Environment. I am also including a summary of research at four different institutions in California by the Dept. of Psychiatry, University of California - Irvine, dated December 16, 1988. These were double blind studies using the analysis of hair . It was found that violent individuals had excessive manganese in a ratio of over six to one to the controls.

14711 Bentley Circle, Tustin, CA 92680 (714) 730-5999

The "manganese marker" was also present in samples collected from males arrested for violent crimes in the State of Louisiana and Texas with 45% and 80% elevated manganese respectively. I also call your attention to the Groote Eyslandt study mentioned by Dr. Donaldson, whereby the highest incidence of murder and violence in the entire continent of Australia is from Groote Eyslandt, which is off of the northeastern coast of Australia. Be advised that Groote Eyslandt is the world's largest manganese mine.

I also ask you to be very cautious with regard to the Ethyl Corporation's manganese MMT compound which Dr. Donaldson believes has a profound effect on fetuses and children because of their blood brain barrier's inability to preclude manganese from entering the brain.

I have met with Ethyl Corporation representatives in an attempt to have them sponsor studies to verify the integrity of the studies made in California. To date, there has been no interest shown by Ethly Corporation in any of this research. I am asking your office to comment as to whether or not the Ethyl Corporation advised you of the Canadian NRC data on manganese toxicity? I am very upset with the Ethyl Corporation concealing the information presently available through scientifically peer-reviewed publications. There is significant evidence that, because of excessive manganese in the environment and its ability to destroy nerve endings, there is a profound effect on humans. More research must be completed.

Any waiver your department grants to the Ethyl Corporation allowing MMT compounds into the environment, will cause irreparable damage to untold numbers of susceptible individuals.

Sincerely yours,

Everett L. Hodges

ELH/mh

Encls.

cc: Mary T. Smith, Director
Mr. Jim Caldwell

A-90-16

UNIVERSITY OF CALIFORNIA, IRVINE

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DEPARTMENT OF PHARMACOLOGY
COLLEGE OF MEDICINE

IRVINE, CALIFORNIA 92717

December 16, 1988

Mr. Everett L. Hodges
14711 Bentley Circle
Tustin, CA 92680

Dear Red:

This letter summarizes the results obtained from the University of California, Irvine (UCI) research project on "Hair mineral levels in a group of violent prisoners and matched controls." The main hypothesis of the research study was that violent individuals would possess hair mineral patterns different from non-violent controls.

In 1985, UCI and California State University, Stanislaus, obtained from the Deuel Vocational Institute in Tracy, California, hair samples from 107 prisoners and 33 guards. Samples were also obtained from 25 control subjects who resided in the Tracy/Stanislaus areas. These samples (n = 165) were drawn equally from Black, Caucasian and Hispanic races. Dr. Rich Haier from the Department of Psychiatry, UCI, in addition, obtained 27 samples from control subjects from the Irvine area.

Dr. Haier coded the samples (n = 192) and sent them to the Honorable Warren Knight, retired Superior Court Judge. Judge Knight retained the codes and mailed the samples to Doctors Data in Chicago, Illinois, for analysis by atomic absorption. The data were examined by us, and the following pattern emerged: Hair samples from violent prisoners, in general, possessed higher levels of manganese. Some violent individuals, in addition, possessed higher levels of lead and magnesium.

The prison volunteers were incarcerated at the facility for extended lengths of time. Therefore, it was argued that these observations could have been artifactual, arising from within the institution.

A second study was proposed in 1986. This study paid particular attention to the length of incarceration of the prison inmates. Thirty samples each were obtained by the San Bernardino and the Los Angeles County Sheriff's Department from prisoners who had been recently arrested for a violent crime. The requirements were that samples could only be obtained from volunteers who had been incarcerated for not more than 30 days. UCI gathered an additional 39 samples from a control group. The samples were analyzed by Doctors Data in Chicago, Illinois.

In this study, the data showed a similar trend in hair mineral levels, namely, high manganese, with high lead and magnesium in violent individuals compared to non-violent controls. On close scrutiny of the data, Rebello, et al., observed that a superior discriminant marker appeared to be manganese levels. In particular levels above 0.70 ppm were observed in violent prisoners with greater frequencies. Because these observations were "post hoc", it was proposed to do a third study to test whether or not manganese levels above 0.7 ppm could be used as a marker for violence.

Sheriff Floyd Tidwell of San Bernardino County agreed to provide 30 hair samples from recently-arrested Caucasian volunteers at the San Bernardino jail. Thirty control samples were collected in the San Bernardino area, 15 from the Fullerton area and 15 from the Irvine area. All samples were matched for age and race. The samples (n = 90) were delivered to Doctors Data for analysis.

The following table summarizes the results:

TABLE I: SAN BERNARDINO JAIL STUDY

Identity	Total	Marked	Percent
Prisoners	30	9	30
Control	60	3	5

Ratio 6:1

TABLE II: DEUEL PRISON STUDY (September 1985)

Identity	Total	Marked	Percent
Prisoners			
Violent Crime/Violent Inmate	32	21	66
Violent Crime/Non-Violent Inmate	34	22	66
Non-Viol. Crime/Non-Viol. Inmate	41	40	95
Prison Guards	33	7	21
Stanislaus Town Controls	25	1	4
UCI Controls	27	3	11
Subtotal Prisoners	107	83	78
Subtotal Controls	85	11	13

Ratio: 6:1

TABLE II: (Continued)

LOS ANGELES and SAN BERNARDINO JAIL STUDIES - September 1986)

Identity	Total	Marked	Percent
Prisoners - Los Angeles	28	11	39
Prisoners - San Bernardino	32	21	66
Control Group	39	6	15
Subtotal Prisoners	60	32	53
Subtotal Controls	39	6	15

Ratio: 3.5:1

TABLE III: CUMULATIVE OBSERVATIONS

Identity	Total	Marked	Percent
Prisoners	197	124	63
Controls	184	20	11

We believe that hair manganese levels may be an important predictor of violence. These observations should be verified using subjects from other areas of the United States.

We are grateful for your financial support and input throughout the study.

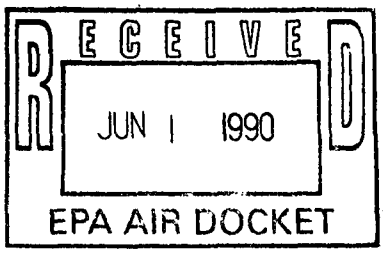
Sincerely,

Tessio Rebello, Ph.D.
Adjunct Assistant Professor

Louis A. Gottschalk, M.D.
Professor

TR:cj

A-90-16



Manganese in the Canadian Environment
NRCC No. 26193.
Associate Committee on Scientific Criteria for Environmental Quality.
National Research Council Canada ISSN 0316-0114
1988. Ed. P. Stokes

MANGANESE AND HUMAN HEALTH

John Donaldson

5.1 INTRODUCTION

Historically, manganese intoxication owes its description to Couper (1837[a,b]) in Glasgow who observed that workers employed in grinding manganese ores, especially braunite, eventually developed masklike faces. The association between brownstone millers and extrapyramidal movements was recorded in a number of countries, but was particularly prevalent in Germany. Von Oettinger (1935), summarizing some 70 cases of manganism, noted that most of such cases occurred in workmen exposed to manganese dust. Muscular stiffness typified by a staccattolike gait and rigid gaze, or retropulsion, were also present.In some cases, hepatic dysfunction was found, suggesting a similarity to hepatolenticular degeneration (Wilson's disease). Intention tremor of the hands and micrography were also distinctive features. Psychic changes, manifested by hallucination and involuntary laughing and crying were present. Cotzias (1958) reviewed several hundred cases of manganese poisoning. He recognized that the neurological symptoms of intoxication were related exclusively to inhalation of massive amounts of manganese dust or fumes when manganese was present in particle size less than 5um. Intoxication occurred only in those workers directly exposed to dust, such as those employed in mining manganese deposits or involved in processing ores. Of special significance was the finding that "old" dusts were less toxic than newly drilled dusts, and that braunite (a mixture of Mn_2O_3 and $MnSiO_3$) was particularly toxic. Because these early observations on (i) particle size; (ii) exclusive neurotoxicity of the aerosol route by dust inhalation; and (iii) dependence of the toxic effects expressed on the oxidation states of the cation went largely

unrecognized for several decades, progress in deducing the underlying phenomenon of manganism was seriously impeded.

5.2 CLINICAL BIOCHEMISTRY

"Locura manganica" refers to madness of acute manganese intoxication, and is particularly prevalent in the mining villages of Chile. Probably the best description of manganism comes from studies of manganese miners in South America. Essentially, acute manganese intoxication is characterized in man by disorientation, memory impairment, acute anxiety, compulsive acts and hallucinations. A remarkable feature in the chronic stage of manganisms is that the extrapyramidal symptoms resemble those noted in Parkinson's and Wilson's disease. Paradoxically, Cotzias et al (1968) and Mena et al (1967) studied the rate of loss of radioactive manganese (^{54}Mn) in manganese miners and found that total body turnover was actually accelerated in healthy workers; this indicated the presence of an expanded, rapidly exchanging manganese pool, as there was no significant difference in total body turnover of manganese dust when normal, nonexposed controls were compared with miners with chronic manganese intoxication. In this regard, Cook et al (1974) demonstrated that elevated urinary concentrations of manganese were a reflection of exposure to the metal and not of the degree of neurological impairment.

There are definite differences in the clinical manifestations of manganese poisoning between patients intoxicated from industrial exposure and miners actively working manganese deposits. Greenhouse (1982), for example, found that manganese-poisoned patients from a factory that he examined did not develop a psychosis, nor did they exhibit rigidity or dystonia.

This investigator also considered that chelation therapy is justified, and may be therapeutically useful in the management of manganism. Because free-radical pathology may be pertinent to the underlying etiology of manganism, consideration should be given to the use of penicillamine as a key therapeutic adjuvant. This agent has proven to be an effective chelator of manganese and, additionally, is an effective scavenger of superoxide radicals.

Cotzias et al (1964) were first to observe an interesting relationship between elevated manganese levels in tissue and deposits of melanin. This finding is of particular importance, because it may relate to the diminished melanin content of the substantia nigra found in both manganism and Parkinsonism; neuromelanin, as distinct from cutaneous melanin, is formed from the nonenzymatic autoxidation of dopamine (Das et al 1978). Recently, Lyden et al (1984) studied the melanin affinity of manganese in vitro using melanin from beef eyes and human hair as well as synthetic dopamine melanin. Manganese was found to have affinity for all three types of melanin, with the highest binding taking place with natural melanin from beef eye. On the basis of these results, as well as in vivo results using whole-body autoradiography, it was concluded that accumulation ofmanganese in melanin-containing tissues is the result of the unique predisposition of manganese for binding sites on melanin. Possible sites may be free carboxyl, hydroxyl or negatively-charged semiquinones. Under conditions of chronic exposure to manganese, accumulation of the cation in pigment-containing neurons of the substantia nigra may result in lesions and movement dysfunction.

A correlation between the incidence of amyotrphic lateral sclerosis (ALS) and the content of manganese in the brain of patients with this neurological disorder has been noted (Yase 1970, 1972). Brain specimens from patients in Guam showed a manganese content 5 to 10 times higher in ALS spinal cord tissue compared with that in control brain tissue. A higher incidence of cases of ALS and/or Parkinson dementia exists among those individuals with a history of having mined manganese deposits on Guam. Recently, Japanese investigators found manganese levels in blood cells of ALS patients were decreased significantly compared with those in patients with other neurological diseases or normal control subjects (Nagata et al 1985).

The ability of manganese to induce insult to the catecholaminergic neurons comprising the nigrostriatal dopaminergic tract and produce clinical symptoms similar to Parkinson's disease, may have considerable relevance towards understanding of the underlying aetiology of this disorder as well as aiding in unraveling the intricacies of neurointoxication by the metal ion. Of more immediate importance, however, study of this relationship seems to indicate that environmental manganese in apparently insignificant and sub-toxic amounts, may play a previously unsuspected and, indeed what can only be described as an insidious role.

In this connection, ongoing investigations now indicate that as a result of initial sub-clinical insult, manganese may contribute towards the accelerated attrition of the neuronal population in strategic areas of the CNS by a process of neuronal age enhancement which may not completely manifest itself until several decades later.In this regard, the senescence of neurons in the substantia nigra of man in early life proceeds at a relatively slow rate of about 1.5% per decade, until later in life (65-84 years) when this process of continuing neuronal attrition is potentiated to over 10% per decade (Mann and Yates, 1983, Mann 1984).This process of continuing disintegration results ultimately in a paucity of dopaminergic neurons in the zona compacta of the substantia nigra. The pathologic aspects become evident after about 75-80% destruction of this area is accomplished. At this point the ability of compensatory mechanisms to maintain physiological integrity is greatly impeded, resulting in the initial manifestations of Parkinsons's disease typfied by muscular rigidity, tremor, and bradykinesia.Thus Parkinson's disease is expressed clinically when only about 20% surviving and viable cells remain intact from the original nigrostriatal neuronal population.

Because of the remarkable compensating ability of dopaminergic neurons, it is clear that any insult to this intrinsically-sensitive and age-vulnerable region of brain in early years would not result in untoward symptoms until a considerable period of time had passed. ie until late mid-life. For this reason, it is considered by some investigators that Parkinson's disease is essentially a disease of exaggerated aging. Current investigations tend to support this concept. In a recent review, Calne and Peppard (1987), have concluded that present knowledge indicates that an increase of normal age-related nigrostriatal degeneration does indeed occur in the elderly members of the population.They thus offer support for the concept that Parkinson's disease, and possibly some other neurodegenerative disorders, may trace their origins to early induced subclinical nigrostriatal damage. In view of the lack of efficient manganese excretory mechanisms, coupled with immature brain permeability barrier development, it is not surprising that younger members of the population comprise a special group particularly susceptible to the peripheral as well as central expression of manganese toxicity. Whether manganese can in fact induce an lesion in discrete and vulnerable compartments of the CNS during the early years and which does not result in neurotoxicity until later stages of development is presently unknown. However, because manganese is an important neurotoxin whose precise mode of nervous tissue and behavioral toxicity is still unknown, it should be considered a prime candidate in studies set up to examine this specific question. Also, the concept that manganese may induce initial sub-clinical neuronal damage which remains dormant for decades until late life when as a result of potentiated senescence changes, it results in a chronic neurologic disorder such as Parkinson's disease, Alzheimer's disease, or amyotrophic lateral sclerosis (ALS) has not yet been adequately addressed.

This subtle aspect of manganism is obviously a critically important area for investigation. The mild lesion which has resulted from an environmental insult to a particularly vulnerable region of brain in early age, when superimposed on a background of normal age-related neuronal compromise and attritiion would thus result in the final expression of clinical symptoms of the disorder. Importance evidence in support of this concept emerges from observations that the powerful Parkinson neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), can induce nigrostriatal damage in people exposed to this agent, but who have not yet developed clinical signs of Parkinsonism (Calne et al 1985).

In view of the preferential sensitivity of catecholaminergic neurons of the basal ganglia to manganese insult, this pivotal finding is of especial relevance to the putative threat of manganese as an environmental neurotoxin. The fact that this possibility has not emerged previously, is due primarily to the pace of current advances embracing innovative neuropharmacologic strategies of nuclear brain imaging in live human subjects at various stages in the aging process.For example, Calne and collaborators (Calne et al 1986) have suggested that etiological mechanisms may play a role by inflicting environmentally-induced damage to the nervous system during the vulnerable prenatal development period. In addition to Parkinsonism, they have extended this hypothesis to include certain other idiopathic neurological degenerative disorders of late life, such as Alzheimer's disease, or ALS. The practical implications of this intriguing hypothesis strongly suggest that search should be undertaken for causal mechanisms linking sub-clinical neuronal damage due to an environmental factor and alteration of the normal cerebral againg process,and that particular attention should be directed towards early rather than late life. A prime candidate

for examination in causal mechanisms linking sub-clinical damage to neurons and an environmental factor and the normal aging process must include manganese. Indeed, as noted elsewhere in this review, such intensive efforts to uncover the reasons underlying the high incidence of behavioral and neurologic disorders in the South Pacific is currently underway.In some regions a correlation between the incidence of such conditions and excessive amounts of environmental manganese, particularly on Groote Eylandt in Northern Australia, has been revealed (Donaldson 1987).For reasons such as these, sources of environmental manganese oxides which are presented to tissues via the aerosol inhalation route are particularly suspect. In view of the reduced selective permeability of the brain in younger members of the general population as well as their reduced ability to excrete manganese, a note of caution concerning the increasing and widespread use in Canada of the antiknock, methylcyclopentodienyl-manganese-tricarbonyl, (MMT), in unleaded gasoline is warranted. Certainly the possibility of initial and insidious insult during the vulnerable developmental period to sensitive areas of the basal ganglia by a delayed process which slowly enhances neuronal attrition over the years to culminate finally in the full-blown expression of neurodegenerative disease must be treated with serious consideration.For this reason, urgently required are well designed studies on primate species, during early, mid and late life and preferably utilizing brain imaging for scrutiny of brain receptors as well as pathophysiological status. In this way it should be possible to determine the effects of low-level aerosol manganese administered during early development for putative neurotoxic as well as neurobehavioral effects at various periods in the life of the animals. A distinct advantage of nuclear brain imaging scanning techniques is that they are conducted in live animals and can thus furnish invaluable data concerning ongoing deterioration in various CNS compartments.

5.3 EXPERIMENTALLY INDUCED MANGANISM

Despite numerous attempts to devise an animal model for study of the underlying pathogenesis of manganese poisoning, such efforts have been beset by many difficulties, not the least of which has been the extensive utilization by investigators of rodent species. Because rodents do not possess melanin pigment in the substantia nigra, use of these species, although generating interesting biochemical data, has not provided a definitive and unifying picture of manganese pathophysiology in humans.

An important aspect of the pathogenesis of manganism relates to the unique ability of the metal ion to undergo changes in oxidation state (Cotzias 1958). The versatility of manganese in undergoing several oxidation changes was suggested by Rodier (1955) to explain the ability of the cation to induce toxicity. Earlier, Von Oettingen (1935) found that manganese salts at 0.2 $mg.kg^{-1}$ was toxic to rabbits, but that 20 $mg.kg^{-1}$ of manganic manganese was required to produce a similar effect. Studies in monkeys (Mella 1924) indicated that prolonged (18 months) injections of manganese chloride could produce choreathetoid movements as well as rigidity and tremor. Aerosol inhalation was used by Van Bogaert and Dallemagne (1945) to replicate in the monkey a clinically useful model of manganism. Extrapyramidal effects were reported by these authors. In other studies (Pentschew et al1963), severe lesions in the pallidus and subthalamic nucleus of monkeys intoxicated with manganese were found.

The neurochemistry of Parkinson's disease is featured by a reduction in the amount of the neurotransmitter dopamine contained in the basal ganglia. Because of the similarity of manganism to

Parkinsonism, experimental investigations of manganese intoxication have tended to focus on the effects of the metal ion on catecholamines in the corpus striatum of various animal species. Depletion of dopamine in the caudate nucleus of monkeys following chronic administration of manganese dioxide is significant (Neff et al 1969), as Gupta et al (1980) observed that monkeys exposed to manganese for 18 months exhibited marked neuronal degeneration concomitant with loss of neuromelanin in the substantia nigra. Other investigators (Mustafa and Chandra 1971; Bonilla and Diez-Ewald 1974) found that manganese loading could decrease brain dopamine in rodents.

Because much of the research on manganese intoxication utilized rodents, which, as noted previously lack brain pigment, progress in pinpointing the mechanism responsible for the metal ion's ability to elicit neurologic dysfunction in man has been slow. No clear-cut interpretation of the phenomenon of manganism has yet emerged. Results of analyses,even within the same experiment, can vary depending on whether samples were obtained during acute, short-term or chronic administration of manganese. Bonilla (1980), for example, found that chronic loading with $MnCl_2$ increased tyrosine hydroxylase activity in rat neostriatum 1 month after treatment, but enzyme activity was decreased after 8 months. Other investigators (Chandra and Shukla 1981) found that manganese treatment produced an initial increase in the content of dopamine (DA), norepinephrine (NE) and homovanillic acid in the corpus striatum of rats, but that this was followed by a period (about midway) when the concentrations were normal, and by a third phase (1 year) when the content of catecholamines and metabolites had declined significantly. It is likely that such discrepancies are mediated by the physical state of manganese in vivo Studies of manganese involvement in plant physiology have revealed that its

function as an essential element in plant nutrition is dependent on the ion's ability to undergo several valency changes. In conditions of manganese excess, the rate of oxidation of Mn^{2+} may exceed the rate of reduction of higher valency species of manganese, and results in necrosis of leafy plants (Kenten and Mann 1950). In humans, the principal effect of neurotoxic insult by manganese may be related to disruption of the endogenous regulatory role fulfilled by different oxidation states of manganese. Indeed, the ability of managanese to assume multi-valence oxidation states endows it with the appropriate prerequisites required of a physiological regulator in situ, (Donaldson and Barbeau 1985, Donaldson 1987). In this connection, examination of some of the physical properties of manganese indicate that manganese is endowed with the necessary characteristics to play an effective role in control of redox homeostasis thereby exerting a powerful influence over regulatory processes concerned with cellular oxidative status. These properties of endogenously localized manganese in body tissues include: (a) an ability to assume multioxidation states, (b) in the Mn^{2+} state it is a powerful scavenger of free radicals such as superoxide anion, (c) in the higher valence Mn^{3+} complexed state it can effectively decompose H_2O_2, and (d), it is a potent inhibitor of lipid peroxidation under in vivo as well as in vitro conditions.

The superoxide anion (O_2-), the hydroxyl radical ($^{\cdot}OH$) and H_2O_2, are highly reactive toxic oxygen species and also powerfully reactive intracellularly. For this reason they possess potent cytotoxic capacity a feature which can represent a serious threat to cellular integrity, especially under conditions where antioxidant defenses are compromised due to nutritive inadequacies, (eg. alpha tocopherol, ascorbate, GSH, or selenium).

Additionally, damage to cellular components particularly biomembranes, can arise due to the formation of lipid hydroperoxides, a process initiated by free radicals. An important source of free-radical production in nervous tissue can arise from the high content of catecholamines present in that tissue. A direct correlation between the capacity of polyphenols for autoxidation and their cytotoxicity towards cultured cells in vitro has indeed been found (Graham et al 1978). The toxic species elaborated are believed to be oxyradicals and semi-quinones which can kill cells through inhibition of sulfhydryl enzymes and reactions with other nucleophilic groups within the cell. Dopamine can both produce free radicals during autoxidation as well as from the formation of neuromelanin precursors. Manganese can enhance considerably the oxidation of dopamine with a concomitant increase in cytotoxic components.

The ability of manganese to oxidise catecholamines is likely due to the formation of Mn^{3+} species which are formed when Mn^{2+} reacts with superoxide anion. In the trivalent state the metal ion can effectively react with the adjacent 3-4-OH groups on dopamine and potentiate its autoxidation with formation of toxic precursors. It has been suggested that this dopamine-oxidation enhancing property of manganese, along with its affinity for pigmented tissues like the nigrostriatal pathway in brain,may explain its ability to induce a toxic lesion in strategic regions of the CNS and initiate movement dysfunction symptoms reminiscent of Parkinsonism (Donaldson and Barbeau 1985).

Manganese also posesses psychotoxic properties and under conditions of acute manganese intoxication, a condition termed, locura manganica, or "manganese madness" can occur. This presents with clinical symptoms, including compulsive behaviors,

reminiscent of those seen during amphetamine-induced psychosis or schizophrenia. In both of these conditions central dysfunction of dopaminergic receptors are believed intimately involved in the pathophysiology of this phenomenon (Donaldson 1987). For this reason it is probable that the hallucinogenic potential of manganese may arise through a special regulatory affinity for neuromebranal processes connected with post-synaptic dopaminergic receptors of the basal ganglia. Certainly, alteration of various neurochemical parameters have been observed during the different phases of manganese neurointoxication, and go a long way to support this contention. For example, manganese given intrapecitoneally to neonatal rats for 15 days, results in increased binding of the dopaminergic antagonist ^{3}H-spiroperidol, to striatal membranes (Seth and Chandra 1984). Increased cholinergic receptor binding was also observed under similar experimental conditions in neonatal rat striatum, an effect which was considered to be related to manganese-induced inhibition of lipid peroxidation in this brain compartment (Donaldson and LaBella 1984). Interestingly, a dose-dependent decrease in high-affinity binding of the cholinergic receptor following incubation of rat brain tissue with catecholamines and manganese has been noted. This effect has been ascribed to receptor destruction or inactivation as a result of membrane damage mediated by oxyradicals or cytotoxic quinones resulting from manganese-potentiated dopamine oxidation.Manganese is a powerful free-radical scavenger and can inhibit lipid peroxidation a process that is known to influence reeptor binding due possibly to alteration of membrane transport processes (eg; Na-K-ATPäse). It

is thus feasible that the psychotogenic potential of manganese ions may develop from their ability to alter critical neurochemical events involved in receptor-neurotransmitter complexes.

In any case, attempts to unravel the intricacies of manganese psychosis, particularly its hallucinatory effects as observed in manganese miners during acute intoxication, are likely to proceed from study of the endogenous function played by the metal ion . It is likely that this role would encompass a regulatory participation in vital physiological events connected with oxyradical formation and in coordinating cellular redox homeostasis. Because of the intimate relationship between manganese and the neurotransmitter receptors in the basal ganglia (Donaldson et al 1974, Donaldson 1987), acute experimental manganese intoxication studies may reveal clues related to the underlying biochemical phenomena involved in the pathogenesis of neurobehavioral disorders. Unquestionably, unraveling of the mode of action and unique characteristics of metallic psychotoxins such as manganese can provide a rich repository of clues leading perhaps to elucidation of the pathophysiology of manganese in discrete CNS compartments.

5.4 CHRONIC MANGANESE NEUROINTOXICATION

A remarkable similarity between chronic intoxication with manganese and Parkinson's disease is apparent from parallel studies.In both disorders, there is a reduction of DA in the caudate nucleus and of NE in the hypothalamus, and there is a similar loss of neuromelanin in the substantia nigra in both conditions (Bernheimer et al 1973). Destruction of DA neurons in the substantia nigra results in depletion of DA in the nigrostriatal pathway, and is associated with locomotor dysfunction in both manganism and Parkinsonism. There is also marked reduction in REM sleep.Additionally, use of l-dopa, the treatment of choice for Parkinson's disease, is also effective in manganism (Mena 1980). The substantia nigra in man, as well as in other primates, is pigmented because of the presence of melanin contained within the cell bodies of dopaminergic neurons in the zona compacta of this brain region.

Studies by Das et al (1978) established that melanin in this area is probably formed nonenzymatically from dopamine by autoxidation. In manganism as well as in Parkinsonism, there is loss of melanin pigment in this area because of chromatolysis of the dopaminergic neurons (Bernheimer et al 1973). In this regard, as noted previously, it has been suggested that the ability of manganese ions to considerably potentiate dopamine autoxidation could result in the generation of free-radical cytotoxins, particularly hydroxyl ($^{\cdot}OH$) radicals. As powerful oxidizing agents, these can initiate the formation of lipid peroxides, disrupting neuronal membrane integrity, and result in cell degeneration.

5.5 REPRODUCTIVE TOXICITY

Non pregnant animals possess an extremely efficient homeostatic mechanism, based redominantly on controlled adsorption and controlled excretion, which maintains tissue manganese levels constant even under conditions of excessive manganese exposure.
But this situation does not prevail during pregnancy, where enhanced concentrations of manganese in blood and tissues can occur because of loss of normal regulatory mechanisms governing homeostasis. The neonatal rat also absorbs manganese readily . In the young rat , intestinal absorption of manganese is about 70% compared with 1-2% in the adult (Mena 1974). This is probably because of the pinocytic activity of intestinal epithelial cells in young animals, which allows particulate manganese oxides to be absorbed, dissolved and ultimately transferred to portal blood. In addition, whereas the blood-brain, intestinal and blood-testes barriers effectively prevent manganese entrance in the adult, a different situation prevails in the fetus, newborn and neonate of rodent species where these barriers are undeveloped. This allows easy penetration of manganese to sensitive sites in brain and reproductive organs. In view of the impermeability of the adult rodent brain to manganese, the neonatal rat, with its ready penetration of manganese, can provide a useful model for study of manganese target-site effects in the central nervous system.

Use of the neonate should obviate many of the difficulties encountered in extrapolation from rat to human when adult rats are employed in manganese intoxication studies.

In adult man, tracer studies for total body of time/activity following administration of $^{54}MnCl_2$ show that intestinal absorption of manganese is 3%; 10 d after exposure about 2% of the dose is retained; and at 50 d total-body retention of the ingested radiomanganese is only 0.2% (Mena 1980). The absorption of manganese is greatly influenced by the iron status of the individual. During iron deficiency, the absorption of iron increases and is accompanied by an increased deposition of manganese in erythrocytes, as well as in the total-body turnover of the element.For example, under conditions of increased iron absorption accompanying iron deficiency anemia or hepatic cirrhosis, the absorption of iron is increased from 11 to 64%, whereas manganese increases to 7.5% from a normal adult value of 3% (Mena 1980). It is thus apparent that the iron status of an individual may greatly influence his susceptibility to the toxic effects of manganese.

During the first 17 d of life in the mouse and rat, there is an absence of excretion of manganese given systemically or via maternal milk. This inability to excrete manganese may account for the marked increase in manganese concentration in the liver and brain observed in neonates at this this time. Dose-related increases in whole-brain manganese of up to 10-fold between 1 and 15 d of life have been found (Cotzias et al 1968). After placental or lactational transfer, fetuses and neonates show a special ability to concentrate manganese in brain compared with adult animals. This retention of manganese, particularly following placental transfer, can continue for some time (Husain et al 1977). This is probably a result of the combined effects of an

immature blood-brain barrier, as well as a diminished ability to excrete manganese during the early neonatal period. In view of the similarity of findings in a considerable number of diverse species with differing types of placentas, it is highly likely that similar effects also occur in humans. Accordingly, in view of the essentiality of manganese for growth and development, it is likely that manganese exposure may be a particular hazard during development (Barlow and Sullivan 1982)

Manganese absorption is greatly accelerated in the newborn compared with the adult. There is also a marked increase in manganese retention in the premature child, indicating relatively poor development of the absorptive barrier, both peripherally and centrally. In view of the enhanced absorptive capacity for manganese, along with the preferential vulnerability and unhindered access to sensitive CNS sites, it is readily apparent that the early development period in man is a particularly susceptible period for the toxic insult of manganese. For this reason, it is of particular importance that, in assessment of risk hazard, priority be given to the insidious neurobehavioral threat that manganese exposure carries for younger members of the population, and for the fetus in utero.

Studies in the adult rat indicate that manganese accumulates preferentially in the liver under normal dietary conditions. During the preweanling period following chronic oral loading with Mn_3O_4, the manganese concentration in the hypothalamus and pituitary exceeds that in the liver. Manganese accumulated during the preweanling period is retained longer than that taken up during the postweanling period (Rehnberg et al $1981_{a,b}$).This avidity of the endocrine and neuroendocrine tissues for manganese in developing animals may be related to reports of reduced fertility in rats given large amounts of manganese (Laskey et al 1982)

Male reproductive development, as assessed by testes weight and sperm count, is delayed by manganese treatment. As well, follicle-stimulating hormone (FSH) and testosterone concentrations are affected. Manganese in excess can therefore represent a threat to the reproductive tissues by virtue of its preferential affinity for the hypothalamus and anterior pituitary (Donaldson et al 1974), and may thus alter the pattern of release of gonadotrophic hormones as well as of testosterone from the testes (Laskey et al 1982). The retardation in male sexual development that accompanies manganese exposure is also exacerbated considerably by iron deficiency.

Histochemical studies indicate that early toxic effects of manganese can alter germinal function of rabbit testes (Chandra et al 1974); the ensuing sterility is permanent, and appears to be related to massive damage to seminiferous tubules. Definitive evidence linking manganese exposure and abnormalities of reproductive function in humans is lacking and is urgently needed. An early study (Penalver 1955) suggested that impotence is a frequent manifestation of manganese poisoning, possibly arising from reduced testosterone secretion (Barlow and Sullivan 1982). A recent study (Lauwerys et al 1985) concluded on the basis of questionnaire data, that there is indication of reduced fertility among the spouses of manganese workers occupationally exposed to manganese fumes. An increased incidence of spontaneous abortions and stillbirths in the wives of manganese workers employed in a number of manganoferrous plants has been noted which, interestingly, was also correlated in severity with both the proximity of industrial exposure to manganese fumes as well as the time worked in the plant (Mandzgaladze 1967).

These observations are of considerable significance since reports of impaired sexual behavior in workers with manganism are, like neurological symptoms, the most commonly reported behavioral symptoms of this disorder (Penalver 1955, Mena et al 1967, Rodier 1955). Observations such as these may offer an example of male-transmitted reproductive dysfunction. Experimentally, manganese can induce a dose-dependent decrease in testosterone in rats, a factor which would account for disturbances in sex functions and testicular changes, including changes in spermatogenesis (Laskey et al 1982).As manganese has a marked affinity for the tissues comprising the hypothalamic-pituitary axis, it is likely that such effects are related to the affinity of manganese for target receptor sites in the median eminence. Intracerebroventricular injection of manganese localises in the hypothalamus, a region where manganese is also located in high endogenous content (Donaldson et al 1974). Additionally, this same route of injection of the metal ion using artificial CSF as a vehicle, results in dose-dependent increases in serum prolactin levels in chronically-canulated rats (Donaldson et al 1974).Hyperprolactinemia is a major factor in the pathogenesis of human infertility. Approximately 25% of cases of secondary amenorrhea are related to elevated prolactin levels. Administration of dopaminergic agonists, such as the ergot alkaloid bromocriptine, which restore normal prolactin levels, results in restoration of endocrine function and fertility.Although the mechanisms underlying such effects are presently obscure, they may be due to the ability of prolactin to inhibit pituitary luetenising hormone releasing hormone receptors (LHRH) in the median eminence, thus altering the physiological function of luetenizing hormone and FSH on sperm maturation.

Consideration of the foregoing, as well as currently related investigations. indicate that manganese may possess the ability to act as a modulator of neuropeptide release, particularly of gonadal hormones governing control of reproductive maturation in humans. For this reason, considerable priority should be given to studies which seek to examine the impact of sub-chronic doses of manganese on spermatogenesis and the influence that impairment of this process plays in relation to male-transmitted reproductive dysfunction. Also, the feasibility of monitoring prolactin levels as a method for determining overexposure in the workplace as well as under conditions of high environmental risk for detecting putative threat to reproductive health in both sexes is a further factor requiring urgent consideration.

5.6 RESPIRATORY DISEASE

The indictment of manganese as a causal agent in pulmonary disease was given considerable credence by the finding that pneumonia was eightfold higher among inhabitants of a small Norwegian town following the opening of a plant which smeltered manganese ore (Lloyd-Davies and Harding 1949). Several other reports of an association between manganese and pneumonia and respiratory disease came from other areas in Europe. The condition known as "metal fume fever", is associated with inhalation of manganese compounds in aerosols or fine dusts. The symptoms include intense dyspnea, high body temperature and ultimately, manganic pneumonia, a form of lobar pneumonia which does not respond to antibiotic therapy.

However, experimentally, inhalation exposure of rabbits or rats did not result in pneumonitis, although fibrosis did occur in association with low hemoglobin and erythrocyte levels (Stokinger 1963; Singh et al 1977). Reports of enhanced susceptibility to infections following manganese exposure alone are scanty, but the powerful superoxide anion scavenging ability of manganese (Donaldson et al 1981) suggests that it could have profound effects on the ability of white blood cells to exert normal antibacterial activity. Superoxide anion (O^{2-}) is produced in phagocytic cells during the respiratory burst, and results in a large increase in oxygen consumption, glucose metabolism and concomitant production of reactive forms of oxygen. These reactive forms are considered necessary for the effective killing of bacteria following phagocytosis. During the initial phase of manganese intoxication, when cells are flooded with excessive manganese, it can be anticipated that the ability of manganese to actively scavenge toxic oxygen species such as superoxide radicals would seriously perturb the ability of phagocytic cells to exert optimal cytocidal activity on ingested microorganisms (Maigetter et al 1976). The impairment of bactericidal activity by manganese could perhaps aid in elucidating the inability of manganic pneumonia to respond to conventional antibacterial therapy.

5.7 TERATOGENICITY

Unfortunately, very few data exist concerning established teratogenic effects of manganese. However, studies which do exist indicate that, even at extremely high doses, manganese does not appear overly endowed with teratogenic potential.Under conditions of manganese deficiency, there is some evidence that the metal may produce skeletal defects in some laboratory species in addition to impairing otolith development in the middle ear (Barlow and Sullivan 1982). Hurley et al (1958) showed earlier that the most prominent effect of manganese deficiency during development is congenital ataxia, which is characterized by loss of

equilibrium, head retraction and tremor. This form of ataxia does not apparently involve a neurological defect. This latter observations underscores our lack of knowledge in relation to manganese metabolism in otolith disorders, particularly those relating to equilibrium dysfunction. In mutant pallid mice, otolith development is impaired in association with genetic changes in manganese metabolism. This congenital form of ataxia results in abnormal otolith development, but the ataxia in the offspring of manganese-deficient animals or of manganese mutants can be prevented by manganese supplementation during pregnancy (Hurley and Everson 1963)

The threshold for electroshock seizures is significantly lower in offspring of manganese-deficient rats following feeding of a manganese-deficient diet (Hurley et al 1961,1963). This relation is important in view of findings by Papavasiliou et al (1979) that whole-blood manganese levels were significantly lower in epileptics than in controls. Also, the blood manganese concentrations were correlated with the frequency of seizures. It. is evident that intensive efforts designed to uncover the relation between dietary manganese during development and neurobehavioral effects, especially hyperactivity, in early childhood are clearly required.

5.8 MANGANESE AND DIABETES

Rubenstein et al (1962) first drew attention to a relation between manganese and carbohydrate metabolism in man. They described a case of a diabetic patient resistant to insulin therapy, but whose blood-sugar levels were amenable to control by oral doses of manganese chloride.The decision to use manganese to lower blood-sugar levels was based on anecdotal reports from African tribal folklore of the usefulness of alfalfa extracts (rich in manganese) for treating diabetes. Oral supplements of cobalt, iron, magnesium or zinc did not

in patients suffering from chronic manganism. In manganese-deficient guinea pigs, abnormal glucose-tolerance curves and decreased utilization of glucose were found (Everson and Shrader 1968). There was also indication of pancreatic hypertrophy which was reversed following dietary manganese supplementation. In certain species of sand rats indigenous to the Israeli desert which have a natural diet rich in manganese, insulin-resistant diabetes was produced when the rats were placed on a low-manganese diet (Shani et al 1972). This condition was reversed when the animals were returned to their normal manganese-rich diet. In the pancreatic beta cell membrane, manganese has been found to block glucose-induced membrane action potentials and to cause cell depolarization. (Dean and Matthews 1970). D-glucose administration also caused increased retention and uptake of manganese (Rorsman et al 1982). Such effects are not simply the results of calcium antagonism, a well-established action of manganese, because the ability of D-glucose to enhance manganese retention was not altered by greatly increasing the extracellular calcium concentration.

Further insight into the relationship between manganese and glucose metabolism was revealed by Hurley et al (1984), who found that rats injected with manganese developed a rapid hyperglycemia and hypoinsulinemia. The blood-glucose and insulin changes correlated with changes in liver and pancreatic manganese, indicating that manganese may play a direct role in insulin release.Altered carbohydrate metabolism, as demonstrated by lower concentrations of plasma glucose, has recently been found in weanling rats subjected to prenatal manganese deficiency (Baly et al 1984). In view of the critical nature of a glucose energy source for the brain, especially in the developing CNS, monitoring of blood-glucose

metabolism in relation to manganese status in pregnanacy and in behavioral changes in offspring is clearly an area that requires careful surveillance. That the effects of manganese on glucose metabolism are not merely academic but can be of critical pathophysiological consequences to man is evident from a report that a patient who had accidentally received a dialysate contaminated with elevated amounts of manganese subsequently developed pancreatitis (Taylor and Price 1982). Though for the present it may be viewed as an emerging area of interest, the effects of manganese on carbohydrate metabolism could indeed represent a real threat to human health by disruption of mechanisms responsible for maintaining glucose homeostasis.

Indeed, pilot studies to explore the feasibility of monitoring for abnormalities of carbohydrate metabolism under conditions of industrial exposure are now warranted.In this regard, a recent study emphasizes that an early clinical sign of manganese intoxication is an elevation of blood sugar (Schunk 1982). It is clear, therefore, that manganese toxicity may not only express itself in altered neurological and neurobehavioral functions, but may also bring about serious distortion: of the physiological homeostatic mechanisms governing the metabolism of cellular glucose. Whether manganese exerts such effects by central neuroendocrine regulation or more directly by interacting with liver glycogen metabolism or beta cells in the pancreas is clearly an area that requires considerable exploration.

5.9 OCCUPATIONAL EXPOSURE TO MANGANESE

Although many difficulties surround the definition of exposure critieria to toxic pollutants under industrial conditions, in the case of manganese this problem is magnified considerably. This situation arises because of a number of factors. For example, arbitrary limits that exist at present were predicated mainly on adult male human exposure, and fail to take account of unique and specialized exposure routes in females. Also preferential

vulnerability of the fetus constitutes a special environmental risk in pregnant women employed in industries where manganese is present in aerosol or particulate matter as a by-product of manufacturing processes. In this connection, reproductive outcomes affecting the fetus may occur because of hormonal disturbances arising from neuroendocrine effects of manganese on the mother, or by direct chemical effects on the fetus. Information on the health risk in human, particularly during development is lacking. But, on the basis of considerable experimental data, there appears little doubt that the human fetus is preferentially susceptible to the neurobehavioral and reproductive toxic effects of the metal ion. Additionally, there is the possibility of developmental disorders arising postnatally in the offspring as a result of exposure from breask milk, or perhaps from contaminants brought home on the parents' clothes. Such effects would differ from the symptoms recorded during adult toxicity, and would almost certainly be more permanent (Barlow and Sullivan 1982).

In considering the range of reproductive hazards to fetal health, it is necessary to take into account that effects on both parents may result in the same end point of reproductive failure, as adverse effects of manganese can be mediated through the male as well as the female. Accordingly, reproductive hazards to the fetus are not confined only to risks arising from direct exposure to manganese, but also to subtle and considerable effects of manganese on complex developmental processes associated with the

normal physiological maturation of male of female germ cells.

Whereas there are considerable difficulties in quantifying health effects to humans based on animal experimental studies, there are sufficient data available to indicate that manganese exposure in the adult emanating from sources outside the factory or mine may constitute a relatively minimal health risk. It must be recognized, however, that children constitute a special group; this is not only because of the immaturity of their systems in dealing with exposure to manganese, but also because of an enhanced absorptive ability.

There are considerable difficulties plaguing the tasks of devising effective health risk criteria standards and instituting definitive limits for occupational exposure to manganese. This situation arises in part because of the apparent lack of correlation that exists between the degree of exposure to the pollutant and the biological response to manganese. Inhalation of manganese dust by Chilean miners resulted in an incidence of only 1-4% of the exposed population and there is no relation between length of time worked in the mine and degree of neurological impairment (Mena et al 1967). Similar findings are evident in a study involving several hundred Yugoslavian ferroalloy workers exposed occupationally to levels of 0.3-20 $mg.m^{-3}$ of manganese alloy. Only a mild degree of neurological impairment (resting tremor) was recorded among the workers exposed to manganese (Saric et al 1977). Such symptoms, which can be indicative of extrapyramidal disorders such as Parkinson's and Wilson's diseases, were recorded in those areas of the plant where only minimal amounts of manganese were present in air, and were not noted in other areas containing moderate and high amounts of the metal. Such data showing negative correlations between the behavioral effects and the content of manganese present environmentally further indicate the inherent complexities of manganese intoxication.

Because the working atmosphere in ferroalloy plants is also polluted with carbon monoxide, carbon dioxide and sulfur dioxide, along with considerable coke and anthracite dust, and because these other pollutants were not considered in the experimental design, rigorous conclusions concerning the overall significance of this study are not possible. An especially puzzling feature of manganism , particularly in mineworkers, has been the failure to find a relation between length of time worked in the mines and the degree of incapacitation from manganese poisoning. A possible explanation for this phenomenon may be the fact that the toxic effects of manganese appear to be closely related to the oxidation state of the metal ion (Donaldson and Barbeau 1985). Thus, exposure to the higher or lower valency states of manganese would depend on the nature of the dusts emanating from particular ores and the type of seams being drilled in the mine. Earlier literature (Cotzias 1958) records that newer drilled dusts were more potent than dusts derived from "older" worked mining deposits.

Experimentally, there are marked differences in the type of biochemical effect obtained by manganese in various oxidation states. Whether such effects are related also to the ability of manganese to affect critical target-tissue sites centrally and peripherally and elicit clinically distinguishable symptoms is not known, but it is an area worthy of further investigation. The diagnosis of manganese intoxication also presents special difficulties, as there are marked differences in the presentation of symptoms between industrially exposed workers and manganese miners actively working ores underground. Although psychosis is a profound feature of overexposure in manganese miners,

this is not the case in factory workers suffering from manganese poisoning. Also, the latter group may not display rigidity or dystonia, whereas these symptoms are common among miners.

In the United States and Japan, the threshold safety limit value of manganese is as high as 5 $mg.m^{-3}$. This contrasts sharply with much lower limits set in some European countries. In Bulgaria, for example, the maximum permissible concentration of manganese as MnO_2 is 0.02 $mg.m^{-3}$; in Poland the the U.S.S.R. it is 0.3 $mg.m^{-3}$; and in Yugoslavia it is set at 2 $mg.m^{-3}$ (Saric et al 1977). However, unlike the USA, such values in the latter countries reflect guidelines rather than standards which are carefully monitored and enforced.

Reports of manganese intoxication have been limited closely, if not entirely, to the workplace, but it is important to recognize that atmospheric levels of manganese under some circumstances can become significant. Manganese levels have been reported to exceed 0.1 $ug.m^{-3}$ in numerous urban areas, and to exceed 0.2 $ug.m^{-3}$ in some American cities (Lown et al 1984). Although figures for Canada are not available, recent environmental data in the U.S.A. indicate emission of relatively high levels of manganese from diesel-powered trucks and automobiles (Pierson et al 1978).
In the United States use of the manganese-based octane booster in unleaded gasoline, methylcyclopentadienyl manganese tricarbonyl (MMT), has now been banned because of increased hydrocarbon emissions. In Canada, MMT has been in use for several years in unleaded gasolines and in terms of energy and economy is efficient in meeting the octane requirements of Canadian vehicle users.

However, the effects of MMT on the Canadian environment, including its health-related risk potential has thus far received only cursory consideration.

The maximal inhalation of manganese from gasoline appears to be in the range of the daily normal adult absorption. As noted previously, however, absorption rates vary greatly with physiological status (e.g. iron deficiency), as well as in pregnant females and young children. Increased manganese levels have been observed in urban-dwelling children, which correlates with increased blood lead levels, thus indicating that blood manganese and blood lead levels could be related to automotive emissions (Joselow et al 1978). Because the inhaltion route of exposure, as in industrial areas and mining operations, is most commonly associated with high risk for expression of the environmental toxicity of manganese, the widespread use of methylcyclopentadenyl- manganese-tricarbonyl (MMT) as an antiknock agent in unleaded gasoline in Canada, can be expected to result in continued increases in airborne levels of manganese oxides arising as combustion products from exhaust emission of vehicles. Similarly, as the elimination of brain manganese follows an extended biological half-time, it is considered that even low levels of manganese following inhalation will result in the deposition in the CNS of greatly increased amounts of the metal ion. Of more importance, current investigations have disclosed that it is not the quantity of manganese arriving at a sensitive target tissue site which provokes damage, but rather that cytotoxicity arises predominantly from the nature of the oxidation potential of manganese; the powerfully-oxidising trivalent species of manganese is especially virulent. Accordingly, it is the valency form rather than the amount of metal ion which dictates the ultimate expression of toxicity in biological tissue.

Thorough comprehension of this critically important aspect of manganese biochemistry thus renders explicable previously puzzling details of the action of this remarkable, albeit biologically-insidious metal ion. In the divalent state manganese is relatively non-toxic and is tolerated cellularly even when present in excessive amounts. However, escalation to higher-valency trivalent species results in a powerful oxidant which even in apparently insignificant amounts can wreak considerable damage to cellular processes (Archibald and Tyree 1987, Donaldson 1987). Another factor obscuring understanding of the toxic profile of manganese has been the paucity of information available on the normal endogenous role played by manganese in tissues. In this connection, the underlying pathogenesis of neurointoxication by manganese is probably related to the unique form of physiological dichotomy displayed by endogenous manganese. In lower oxidation states (Mn^{2+}) manganese can effectively scavenge superoxide radicals and is a powerful antioxidant. This property may endow manganese with a potential superoxide-scavenging role in regions of high oxidative activity of brain like the putamen of the basal ganglia, where both manganese and another powerful antioxidant metal ion, selenium, are present in extremely high amounts.

In high oxidation states (Mn^{3+}) the metal ion is a powerful oxidant a form in which it can display its toxic profile by catalysing the oxidation of biologically critical substrates such as catecholamines, unsaturated fatty acids, and glutathione; processes which are accompanied by the elaboration of toxic oxyradicals. Recent studies on the clearance of labelled manganese chloride from brain following inhalation (Newland et al 1987), intriguingly, have revealed that inhaled manganese may not exhibit untowards effects until some considerable time after exposure.

A principal feature of the aerosol route of exposure is that manganese is able to pass directly into the bloodstream. Accordingly, by circumventing hepatic and gastrointestinal clearance mechanisms, procedures which result in the elimination of 97% of orally ingested manganese, the content of inhaled manganese increases greatly in the cirulation. Of equal importance, it can also remain present within the pulmonary aleveoli for extended periods of time thus providing a reservoir for sustained release over prolonged time periods to cerebral tissue. While systemically-administered manganese can clear the head area in a few weeks, accumulated deposits of manganese in the lung are released only gradually thus leading both to enhancement of brain tissue levels as well as prolonging the residence time of manganese in that tissue. Pharmacokinetic studies such as these clarify conclusions arising from earlier investigations in manganese miners which indicated that manganism can persist for a considerable period of time after exposure,even when the clearance of manganese from body stores has apparently ended (Cotzias et al 1968, Mena et al 1967). As noted previously, it is not the amount of metal ion which has gained access to sensitive target areas in the brain which results in toxicity, but more likely the oxidation state in which it is ultimately deposited.
Under conditions of manganese neurointoxication it is to be expected that exogenous manganese would be distributed throughout the CNS. In this way, therefore it would be anticipated that lesions provoked by its neurotoxic expression would be generalized and thus inflict damage in various brain areas rather than confined predominantly to dopaminergic neurons in basal ganglia and nigrostriatal tissue.This would explain the ability of manganese to induce a selective lesion in a discrete area like the substantia nigra as most likely due to the nature of the biochemical milieu present in this highly-oxidative region which can induce the oxidation of innocuous lower-valency Mn^{2+}, to the

cytotoxic Mn^{3+} species. Since Mn^{3+} is a highly-reactive manganese oxidant (Donaldson and Barbeau 1985, Archibald and Tyree 1987), accumulation of Mn^{3+} in the dopamine-rich nigrostriatal tract could result in the potentiated autoxidation of dopamine (Donaldson et al 1981), a procedure which results in release of toxic oxygen species. This situation would result in neurodegenerative effects arising from oxyradical damage per se by directly attacking sensitive sites on neuronal membranes, or as a result of the initiation of lipid peroxidation. Injury to catecholamine neurons due to the by-products of catecholamine oxidation could be enhanced as a result of the concomitant impairment of the neuronal protective system (glutathione, NADPH,SOD) arising from the ability of manganese to deplete cellular thiols.

5.9.1.

MANGANISM AND THE"GROOTE EYLANDT SYNDROME"

Manganism has recently also been described in a neurological ethnic complex in the Northern Australian Territories (Kilburn, 1987). The aboriginal inhabitants of Groote Eylandt in the Gulf of Carpentaria display unusual clinical conditions, including neurologic entities with prominent motor-neurone features, connective tissue disorders, congenital malformations, and psychiatric excitement. The occurrence of such disorders comprise part of the components now termed "Groote Eylandt Syndromes", and are the subject of increasing concern by community and health workers in this region (Donaldson 1987, Kilburn 1987).

Groote Eylandt is a relatively large island located in the western end of the Gulf of Carpentaria at 137 degrees east longitude and 14 degrees south, some 50 K off the east mainland coast of the Arnhem Land Aboriginal Reserve.

The Island is located in the same geographical region (140°) where motor-neurone disease syndromes, and Parkinson dementia of the Western Pacific, including Guam, Kii Peninsula, Western New Guinea, are found in highly significant numbers.Such factors strongly indicate that an environmental agent(s) is a likely contributor to the etiology of such neurobehaviorally-bizarre phenomena.Manganese oxide ores, such as pyrolusite and cryptomelane are actively mined on Groote Eylandt and manganese dioxide dust is present in the air and deposited throughout the terrain. Mining operations have been conducted for some time and the potential importance of the studies on Groote Eylandt is related to the fact that the native population has been exposed to a known manganese-bearing ecology for several decades. Several other factors unique to the aboriginal residents of the Island may contribute to the toxic expression of manganese and its possible relation to the high incidence of health problems manifest in the local population.

It is firmly established that the CNS in the fetus and neonates is preferentially vulnerable to toxic insult due to a marked permeability of the blood-brain-barrier during development. Also, the excretory mechanisms, an important mechanism of regulating manganese homeostasis and which rapidly eliminate this metal ion in the adult, are poorly developed in infants and children. Along with chronic iron deficiency, another factor which could favor increased manganese penetration to toxic sites in the body, is that the calcium content in the water and soil on Groote Eylandt is extremely low. Studies of foci of high incidence of motor-neuron disorders in the Western Pacific has also revealed unusually low concentrations of calcium and magnesium in soil and water in the area. Certainly, in combination with excessive amounts of metals such as manganese, selenium and aluminum, deficiencies of an

essential mineral such as calcium can greatly impede critical absorptive mechanisms which play an important role in regulation of mineral homeostasis. This factor, along with abnormal mineral metabolism, can result in preferential transport to discrete compartments of the brain of neurotoxic metals which would normally be excluded. As discussed earlier, the pathophysiological effects of manganese in brain is not necessarily the presence of excessive amounts of the ion, but rather the presence of selectively toxic higher valency species of manganese which have the inherent property of inducing selective neuronal injury in discrete, and mangano-compatible regions like the nigro-striatal dopaminergic tract.

During recent investigations on Groote Eylandt several former mine-workers have been found to have a neurological disease complex with upper motor-neurone and cerebellar signs which were associated with very high (600-700 nmol/l) blood manganese levels (Cawte et al 1987).The expression "bird disease", so termed due to the peculiar strutting leg movements of those afflicted, has been applied by the local inhabitants to those aboriginals in the community affected by this syndrome. The dysfunction in gait is reminiscent of the phenomenon of demarche en pied de coq, or "cockwalk" in which the patient tends to use small steps and simulataneously tends to rotate the heels, elevating them outward and progressing without pressing on the flat of his feet.
Similar movements were recorded following neurological investigations of ten patients at manganese mines in Northern Chile (Barbeau 1984). Although data on the psychiatric manifestations of this disorder among the aboriginal inhabitants is not available, it is significant that the record of arrests and incarcerations of the native population on Groote Eylandt is, per capita, the highest in the country.

5.10 CONCLUSIONS

It is apparent from this brief review of some of the more pertinent observations concerning the assigned and putative toxic effects of manganese as an atmospheric and industrial pollutant that considerable gaps exist in our knowledge of the real and implied threats of this metal ion to human health. Perhaps the most disturbing note, apart from the paucity of data actually available on the subject "manganism" itself, is the difficulties involved in diagnosing the condition in the occupational health setting. There are many areas which require intensive investigation in regard to ultimately obtaining the most effective diagnostic and therapeutic means for treating intoxication by manganese. With respect to occupational health standards, it is suggested that future research endeavors on manganism should be concerned with areas which have early practical application., Some areas which can be assessed as requiring priority exploration in the laboratory and workplace are as follows:

(i) A note of especial concern must be voiced concerning a rapidly accruing new body of evidence linking chronic neurodegenerative conditions to the suspicion that these disorders may only manifest themselves as a result of early sub-clinical lesions which may be initiated by environmental neurotoxins such as manganese. By initially inducing a primary lesion in a strategic CNS compartment during neonatal or even prenatal conditions which remains dormant and undetected for several decades, by continuing neuronal attrition it ultimately produces a chronic brain disorder in late life, such as Parkinsonism, ALS, or Alzheimer's disease. Because infants and young children comprise a special group with immature systems for dealing with toxins, studies in primates which seek to determine if low doses to manganese to young animals can eventually

lead to enhanced compromise and attrition of the neuronal population in later years are urgently required. Since manganese is capable of also inducing an acute syndrome similar to schizophrenia, neurobehavioral studies which focus on the psychiatric manifestations of the metal ion are an additional priority.

(ii) In view of the potential hazard of manganese for human reproduction, monitoring for reproductive toxicity in the industrial setting, by study of case histories of workers is suggested. This should include reproductive history; menstrual cycle irregularities; stillbirths; birth weights; and estrogen and testosterone levels. Such studies would aid in assessment of problem areas in the plant.

At the laboratory level, considerable study is required of the physiopathological effects of manganese on the reproductive process, and this is especially true at the level of neuroendocrine control of male and female germ cells.

(iii) The possible usefulness of blood glucose and glucose tolerance testing under industrial exposure conditions as an indication of manganese exposure requires examination.

In the experimental area, studies set up to explore the role of manganese in the pancreatic B-cell control of glucose homeostasis as well as at the central level are necessary, in order to deduce the toxic potential of the metal ion in disorders of glucose metabolism.

(iv) Under industrial conditons, the presence of subchronic infections in workers which are refractory to antibiotics should be recorded as a possible indicator of overexposure to the pollutant manganese.

Experimentally a need exists for studies to explore the effect of manganese exposure on cellular defence processes, especially those concerned with biochemistry of phagocytosis.

(v) Information is required concerning the relationship between nutritional status and the clinicopathologic effects in animals poisoned with manganese, in order to determine its clinical application in studies designed to set up meaningful exposure level limits for industrial workers.

(vi) At the neurobehavioral level, studies of the psychiatric manifestations of manganese are urgently required.

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Manganese Neurotoxicity: Possible Clues to the Etiology of Human Brain Disorders

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INTRODUCTION

Manganese possesses appropriate criteria for inclusion in a volume relating metal ions to neurology and psychiatry, since both neuropsychiatric behavioral disturbances and bizarre psychoticlike phenomena as well as profound neurological dysfunction are associated with exposure to manganese-laden dusts. Although ostensibly it would appear that study of an agent capable of provoking both psychiatric and extrapyramidal disturbance would generate a rich repository of clues by which to uncover the underlying neurochemical bases for such disorders, the yield realized, in terms of advancing knowledge of the clinicopathologic features of this phenomenon, unfortunately has been disappointingly meager. Indeed, perhaps this is not too surprising a finding since early reports on manganism considered it to represent a clinical entity indistinguishable from idiopathic Parkinson disease. It now appears, however, that the neurological sequelae of manganese intoxication may represent part of a category of abnormal involuntary-movement disorders rather than a specific neurological entity [Barbeau, 1984a,b].

Historically, manganese intoxication owes its descriptions to Couper in Glasgow who observed that workers employed in grinding manganese ores, especially braunite, eventually developed masklike facies [Couper, 1837a,b]. This link between brownstone millers and extrapyramidal movements was recorded from a number of countries in Europe but was particularly prevalent in Germany. Summarizing some 70 cases of manganism, Von Oettingen [1935] noted that most such cases occurred in workmen exposed to manganese dust. Rigid gaze and muscular stiffness, typified by a staccatolike gait or retropulsion, was also prevalent. Hepatic dysfunction was present in some cases, suggesting a similarity to hepatolenticular degeneration (Wilson disease). Intention tremor of the hands and micrography were also commonly observed. Psychic changes, manifested by hallucinations and involuntary laughing and crying, were present also. Cotzias [1958] reviewed

several hundred cases of manganese poisoning. He astutely recognized that intoxication was related exclusively to inhalation of massive amounts of manganese dust or fumes when manganese was present in a particle size less than 5 μm. Also, intoxication occurred only in those workers directly exposed to dust such as those employed in mining manganese deposits, or who were involved in processing ores. Of especial significance was the finding that "old" dusts were less toxic than newly drilled dusts and that braunite (a mixture of Mn_2O_3 and $MnSiO_3$) is particularly toxic.

These critical observations on (1) particle size, (2) exclusivity of the aerosol inhalation route by generation of dust, and (3) recognition that the toxic effects expressed were dependent on the oxidation states of the cation were unfortunately ignored by a majority of researchers for several decades with the result that progress in deducing the underlying phenomenon of manganism as expressed both clinically and biochemically has been seriously impeded.

Probably the best description of manganism emanates from the studies of manganese miners in South America. *Locura manganica* refers to the madness of acute manganese intoxication and is particularly prevalent in the mining villages of Chile. A description of the experimental production of manganism was recorded by Mella [1924]. Essentially, acute manganese intoxication is characterized in man by disorientation, memory impairment, acute anxiety, compulsive acts, and hallucinations. In the chronic stage of manganism, a remarkable feature is that the extrapyramidal symptoms resemble those noted in Parkinson disease and more so to those of Wilson disease. Paradoxically, Cotzias et al. [1968] and Mena et al. [1967] studied the rate of loss of radioactive manganese (^{54}Mn) in chronic manganese poisoning and found it normal. This observation has long awaited confirmation by other investigators.

Parkinson disease is characterized biochemically by a reduction in 3,4-dihydrophenylethylamine (dopamine) in the caudate nucleus [Ehringer and Hornykiewicz, 1960]. For some years the disease has been relatively successfully treated by administration of the precursor of dopamine, L-3,4-dihydroxyphenylalanine (L-dopa). This compound, unlike dopamine, is able to cross the blood-brain barrier. Of considerable importance to an understanding of the underlying mechanism causing Parkinson's disease is the observation by Bernheimer et al. [1973] that the caudate nucleus in one case of manganese intoxication examined is also deficient in dopamine. It is especially pertinent that the patient had a previous record of extremely elevated serum manganese levels. Norepinephrine in the hypothalamus was also severely reduced. This striking biochemical similarity between manganism and parkinsonism is clinically supported by the work of Mena and co-workers [1970]. These investigators have found that administration of L-dopa to miners suffering from the hypokinetic form of chronic manganese poisoning results in a striking reduction or disappearance of rigidity and hypokinesia.

Greenhouse [1982], however, in studying several cases of manganism arising from occupational exposure, did not obtain favorable results with dopa and, despite contrary evidence by others [Mena et al., 1970], considers that chelation therapy is justified and may be therapeutically useful in management of manganese poisoning. Greenhouse [1982] notes also that the manganese-poisoned patients he has examined did not develop a psychosis nor did they exhibit rigidity or dystonia. Barbeau et al. [1976] consider that the symptoms in manganese miners are more reminiscent of dystonia than those seen in Parkinson disease. Such apparently conflicting factors led other investigators [Greenhouse, 1982] to conclude that manganism may be composed clinically of two major divisions—a group of dopa respondents showing increased tone and rigidity, and a second group displaying a bradykinetic form that is not helped by dopa therapy. Barbeau [1984b] has also presented convincing clinical evidence that manganism is essentially a mixture of extrapyramidal bradykinesia and dystonia. Results such as these would certainly provide a rational explanation for conflicting reports on therapeutic intervention. In this regard Greenhouse's [1982] suggestion that penicillamine should be considered as a key therapeutic adjuvant in manganese intoxication is worthy of emphasis. We have found this agent an excellent manganese chelator experimentally; in addition, it is an effective scavenger of superoxide radicals, and such vectors may play an important role in the pathogenesis of manganism.

An interesting relation between elevated manganese levels and melanin deposits in tissue has been noted by Cotzias et al. [1964]. Moreover the melanin content in the substantia nigra of Parkinson patients is diminished. It is therefore conceivable that manganese may influence the formation of both dopamine and melanin, since for both compounds the precursor can be L-dopa, and neuromelanin in the substantia nigra is thought to arise from nonenzymatic autoxidation of catecholamines, principally dopamine. Yase [1970, 1972] has noted a correlation between the incidence of amyotrophic lateral sclerosis (ALS) and the content of manganese in the brain of patients with this neurological disorder. Analyses of fresh brain specimens from patients in Guam showed a manganese content 5–10 times higher in ALS spinal cord tissue than in similar CNS tissue in control cases. There was also a higher incidence of cases of ALS and/or Parkinson dementia among those individuals with a history of having mined manganese deposits on Guam.

EXPERIMENTALLY INDUCED MANGANISM

Although a veritable plethora of models of manganese encephalopathy abound in animals and purport to reveal a clinical-pathologic entity reminiscent of that in human intoxication, close scrutiny of such claims unfortunately reveals little information casting illumination on the underlying intricacies of this phenomenon. Among a number of critical factors delaying

interpretation of results of animal experimentation in recent years has been the oversight by many investigators to fully appreciate key factors painstakingly uncovered by the pioneering and diligent efforts of earlier investigators. In this regard Cotzias [1964] had noted the difficulties involved in devising an animal model for manganism and attributed this to the use of species—primarily rodents—lacking melanin pigment in their substantia nigra. Although some interesting biochemical data have been generated from neurointoxication studies using rats, a definitive picture of manganese pathophysiology has yet to be revealed.

Cotzias [1958] in his brilliant review of the field considered that an important aspect of the pathogenesis of manganese must relate to the unique ability of the metal ion to undergo changes in oxidation state. Rodier [1955] had also suggested a relation between oxidation of manganese and its ability to produce toxic symptoms. Earlier workers [Von Oettingen, 1935] found that manganese salts at 0.2 mg/kg were toxic to rabbits but that 20 mg/kg of manganic manganese was required to produce a similar effect. Early studies in monkeys [Mella, 1923] indicated that prolonged (18 months) injections of manganese chloride could produce choreathetoid movements as well as rigidity and tremor. In an attempt to duplicate the natural exposure route, aerosol inhalation was used by Van Bogaert and Dallemagne [1945] to duplicate in the monkey a clinically useful model of manganism. Extrapyramidal effects were reported by the investigators. This study has been subsequently widely cited in favor of a manganese action primarily on the basal ganglia. Close scrutiny of this experiment (published in French) reveals that only one monkey was used. Further, this animal was exposed numerous times to fumes emanating from a melange of sodium perchlorate, ammonia, and manganese dioxide following their electrical decomposition. Thus, the clinical and anatomical effects described may have arisen in fact from the ammonia and chlorine fumes generated by this method as well as to attributed manganese dioxide effects! In more recent studies [Pentschew, 1963; Pentschew et al., 1963], severe lesions in the pallidum and subthalamic nucleus of monkeys intoxicated with manganese were found.

A particular feature of the neurochemistry of Parkinson disease is a reduction in the amount of the neurotransmitter dopamine in the basal ganglia. For this reason, experimental investigations of mangagnese intoxication have tended to focus on the effects of the metal ion on catecholamines in the striatum of varius animal species. Neff et al. [1969] demonstrated depletion of dopamine in the caudate nucleus of monkeys following chronic administration of manganese dioxide, and others [Mustafa and Chandra, 1971; Bonilla and Diez-Ewald, 1974] reported similar changes in rodent brain. Firm conclusions from studies such as these which employ large amounts of manganese over a prolonged period are tenuous because administration of metals ions in excess can actually result in deficiencies of other, unrelated essential elements. Thus, the effects observed are not directly

attributable to the experimental ion but to the metal deficiency state attained.

Because of the lack of a suitable experimental model of manganese encephalopathy, as well as the reluctance of investigators to appreciate the contribution of the multioxidation states of manganese to the expression of the metal ion toxicity, progress in pinpointing the mechanisms responsible for its ability to elicit neurologic dysfunction in man has been slow. Thus far no clear-cut interpretation of the phenomenon of manganism has yet emerged. Another problem has been the variability of results. Results of analyses even within the same experiment can vary depending on whether samples are obtained during acute, short-term, or chronic intoxication by manganese. Bonilla [1980], for example, found that chronic loading with $MnCl_2$ increased tyrosine hydroxylase activity in rat neostriatum 1 month after treatment but that enzyme activity was decreased after 8 months. Other investigators [Chandra and Shukla, 1981] found that manganese treatment produced an initial increase in the content of DA and NE, and homovanillic acid in the corpus stratum of rats, but was followed by a period about midway when the concentrations were normal and by a third phase (1 year) when the content of catecholamines and metabolites had declined significantly. From such perplexing results it is apparent that the intricacies of manganese neurointoxication trace their basis to the *duration* of the experiment and the biphasic effect of the metal ion in both stimulating and depressing monamine metabolism. Because of the disparity in results both within and between experimentation in the laboratory of a number of researchers, it is likely that such discrepancies are mediated by the physical state of manganese *in vivo*. For example, early studies of manganese involvement in plant physiology have revealed that its function as an essential element in plant nutrition was dependent on the ion's ability to undergo several valency changes. Under conditions of manganese excess, the rate of oxidation of Mn^{2+} ions may exceed the rate of reduction of higher-valency species of manganese, resulting in accumulation of higher oxides of manganese with resultant necrosis of leaf plants. In humans, the primary effect of neurotoxic insult by manganese may be related to disruption of a normal regulatory role fulfilled by different valency states of manganese.

During ongoing investigations related to the neurotoxicology of manganese we have been impressed by the similarity in behavioral and neurochemical events elicited by manganese and the potent neurotoxin, 6-hydroxydopamine (6-OHDA). Behavioral similarities evoked by such diverse agents may be due to the ability of manganese to promote the generation of 6-OHDA in nervous tissue, or to a mechanism, common to both agents. We have evidence that this similarity may be due to the ability of manganese to potentiate dopamine autoxidation with the concomitant production of hydrogen peroxide (H_2O_2) and free radicals such as superoxide anion ($O_2^{\cdot}$), and the hydroxyl radical ($\cdot OH$). Production of such toxic free radicals is

believed to be the method by which 6-OHDA expresses its neurotoxicity, and the ability of manganese and 6-OHDA to act in this way could explain their similarity of action in the CNS, including the ability of manganese to induce neuronal degeneration. Elucidation of the neurochemical events by which manganese induces its neurotoxic profile in brain can also provide important clues to the mechanism underlying the pathogenesis of neurodegenerative diseases. The possibility that manganese dyskinesias are produced owing to the potentiated autoxidation of dopamine by manganese is supported by Graham and collaborators [1978]. These investigators have demonstrated that cytotoxic products are indeed produced during autoxidation of dopamine and that the degree of autoxidation of a particular catecholamine was closely linked to its cytotoxicity against neuroblastoma cells.

Recently [Donaldson et al., 1981, 1982] we have suggested that the ability of manganese ions to considerably enhance autoxidation of dopamine can result in the production of free radicals, particularly hydroxyl radicals, ·OH, and semiquinone species. Both species possess considerable cytotoxicity [Graham, 1978, 1984] and in discrete brain compartments like the substantia nigra could wreak considerable havoc among catecholaminergic nerve cell bodies localized there. Since this region of brain contains the pigment neuromelanin which is derived from dopamine nonenzymatically [Das et al., 1978], neurointoxication by manganese may be expected to considerably augment dopamine oxidation and concomitant formation of cytotoxins.

That the products of dopamine oxidation (free radicals, semiquinones) are responsible for the degeneration occurring in the nigrostriatal tract in manganism is difficult to establish experimentally. However, *in vitro*, dopamine oxidation by-products have been shown to be toxic to neuroblastoma cells in culture and can alter binding of central neurotransmitter receptor ligands [Graham, 1984; Donaldson and LaBella, 1984]. High-affinity receptor binding, which can be useful in detecting damage to specific neuronal circuits, was decreased following incubation with dopamine and manganese under conditions favorable for oxidation of the catecholamine (Tables 1, 2). Both increasing the amount of dopamine and the duration of incubation led to increased inhibition of binding of the cholinergic neuronal radioreceptor ligand [^{3}H]QNB. The inhibitory effects of Mn-enhanced dopamine autoxidation by-products on [^{3}H]QNB binding could be reversed by addition of catalase, indicating the likelihood of active oxygen species being involved in the kinetics responsible for the decrease in neurotransmitter receptor binding.

The foregoing results may indicate that long-term therapy with L-dopa, which leads to dopamine enhancement in the striatum, could also lead to increased production of neurotoxins with subsequent neurodegenerative effects being masked by temporary alleviation of extrapyramidal symptoms. However, catecholamines in high concentration can also exhibit powerful free-radical scavenging ability and may conceivably exert an antioxidant

TABLE 1. Inhibition of Cholinergic Receptor (^{3}H-QNB) Binding by Dopamine Oxidation Products

Tissue	Dopamine concentration (mM)	Specific bind (cpm/mg)	Percent change
Whole brain	Control	7,448 ± 383	–
Whole brain	1	6,476 ± 202	− 13 ± 1.9*
Whole brain	5	6,209 ± 398	− 17 ± 4*
Whole brain	10	5,266 ± 562	− 29 ± 3**
Whole brain	20	4,999 ± 265	− 33 ± 6**

Control and test samples (14) were incubated for 1 h at 37°C in beakers containing the dopamine concentrations indicated along with manganese (50 μM) and Tris buffer, pH 8.0. Control beakers incubated in absence of manganese.
*$P < 0.05$; **$P < 0.01$.

TABLE 2. Effect of Dopamine Oxidation By-products at Varying Time Intervals on ^{3}H-QNB Binding in Rat Whole Brain

Time (min)	Control (cpm/mg)	Sample (cpm/mg)	Percent change
0	6,958 ± 450	6,841 ± 432	−1.7 ± 0.8
20	7,118 ± 300	6,460 ± 425	−9.3 ± 2.5
60	6,676 ± 235	5,474 ± 467	−24.6 ± 2.2**

Control and test samples (12) were incubated for the time periods indicated in 10-ml beakers containing dopamine (20 mM), manganese (50 μM), and Tris buffer, pH 8.0. Control beakers were incubated in the absence of light.
**$P < 0.01$.

role under *in vivo* conditions (Cohen, 1983). A major free-radical precursor elaborated during catecholamine oxidation is hydrogen peroxide. The hydroxyl radical is a by-product of H_2O_2 and has been strongly implicated in the neuronal degeneration induced by 6-OHDA. It may also be a component in pathological processes such as the rapid brain aging seen in Down syndrome, in Alzheimer disease, and in inducing nigrostriatal damage in parkinsonism (Cohen et al., 1976).

CHRONIC MANGANESE NEUROINTOXICATION

In man, as well as in primates, the substantia nigra is pigmented owing to the presence of melanin contained within the cell bodies of dopaminergic neurons in the zona compacta of this brain region. Studies by Das et al. (1978) have established that the melanin in this area is formed nonenzymatically from dopamine by autooxidation. In manganism, as well as in Parkinson disease, there is loss of melanin pigment in this area owing to chromatolysis of the dopaminergic neurons (Bernheimer et al., 1973). Destruction of DA neurons in the substantia nigra leads to profound depletion of DA in the nigrostriatal pathway. Loss of striatal DA in manganism, as

well as in parkinsonism, is associated with locomotor dysfunction in both conditions. These clinical symptoms respond in both disorders to treatment with L-dopa, the DA precursor [Mena et al., 1967]. Until recently, the observed melanin loss in human substantia nigra has not been examined experimentally. Gupta et al. [1980], however, have recently found that monkeys exposed to manganese for 18 months exhibit marked neuronal degeneration with loss of neuromelanin in the substantia nigra. An explanation of the degenerative effects induced by manganese in the substantia nigra may derive from the following considerations:

1. Neurodegeneration in the substantia nigra may be related to the ability of this region to generate excessive amounts of H_2O_2. The ability of manganese to induce selective focal lesions in the substantia nigra of man [Bernheimer et al., 1973] and primates [Gupta et al., 1980] may be related to the high content of oxidative enzymes in this region [Cote and Fahn, 1969]. The propensity of this region to elaborate large quantities of H_2O_2, through oxidative deamination of catecholamines by MAO, is indicated by the data of Ambani et al. [1975], who found that the highest peroxidase activity in human brain was located in the substantia nigra. Since divalent manganous salts are oxidized to higher-valency Mn^{3+} or Mn^{4+} forms in the presence of peroxidase, phenols, and H_2O_2 [Kenten and Mann, 1950, 1957], an intriguing possibility to consider is that the milieu within the substantia nigra favors the escalation of exogenous divalent manganese to higher-valency forms. Under conditions of manganese neurointoxication, exogenous manganese would be distributed throughout the CNS, so it would be expected that lesions arising from the neurotoxic effects of this metal ion would be generalized rather than focal. However, if the constituents within a particular region (e.g., high H_2O_2, peroxidase) favor the formation of higher-valency manganese, then the ability of the ion to induce a selective lesion can be understood. Higher-valency manganese, probably Mn^{3+}, can potentiate the autoxidation of dopamine, NADPH, or glutathione with concomitant production of free radicals [Donaldson et al., 1981]. Accumulation of Mn^{3+}, in the dopamine-rich substantia nigra of man could thus result in uncontrolled proliferation of active oxygen species. Since neuromelanin is formed nonenzymatically from dopamine [Das et al., 1978], enhanced generation of cytotoxic orthoquinone precursors of neuromelanin would also result [Graham, 1978; Graham et al., 1978]. Under these circumstances, excess free radicals would potentiate lipid peroxidation and result in tissue destruction. Since Mn^{3+} enhances the oxidation of NADPH and glutathione [DeRycker and Halliwell, 1978; Curnutte et al., 1976], the neuronal antioxidant defense system would also be weakened as optimal activity for the key scavenger of H_2O_2 and lipohydroperoxides, glutathione peroxidase, is tied to availability of these substrates. Direct injury to catecholamine neurons by the by-products of catecholamine oxidation is, however, unlikely without concomitant impairment of the neuronal protective system (glutathione,

NAPDH, SOD). Added significance must then be taken of manganese's ability to deplete cellular thiols [Eriksson and Heilbronn, 1983] and thus by implication disrupting critically important enzymes such as adenylate and guanylate cyclase, which are exquisitely sensitive to changes in cellular redox potential. Accordingly, generation of toxic oxygen species as a result of accelerated oxidation along with impaired neuronal protective mechanisms may underlie the catecholaminergic degeneration seen at autopsy in manganese poisoning.

2. The lesions that arise in the substantia nigra result from a "dying back" phenomenon. In rats the hypothalamus is a primary target in the acute stages of manganese neurointoxication [Donaldson et al., 1982]. As manganese accumulates in the hyothalamus, its ability to decompose H_2O_2 to nontoxic products (reaction II) would be competitively impaired by reaction III (see below). In the latter reaction, the combination of H_2O_2 and elevated Mn^{2+} provides the necessary conditions for generating the highly toxic hydroxyl radicals which have been suggested as important cytotoxins in neuronal degeneration [Graham, 1978; Graham et al., 1978].

This manganese-induced toxicity, mediated by hydroxyl radicals, in the hypothalamus could exert an indirect cytotoxic effect on the substantia nigra, since axons coming from the nigra impinge on the lateral hypothalamus. The nigrostriatal system consists of neurons whose perikarya are located within the zona compacta of the substantia nigra. The dendrites of such neurons project into the zona reticulata of the nigra and their axon bundles pass through the lateral hypothalamus prior to entering the corpus striatum, where they undergo extensive terminal ramifications. Hydroxyl radicals, generated from H_2O_2 by Mn^{2+} accumulated in the hypothalamus, may inflict damage on the nigrostriatal axons as they pass through this region. This could result in a "dying back" phenomenon resulting in nigral degeneration. Recent electrophysiological evidence supports this concept. Maeda and Mogenson [1981] found that enhancement or suppression of dopaminergic neuronal activity in the zona compacta of the substantia nigra of the rat was obtained following electrical stimulation of the lateral hypothalamus. This suggests that the latter region may exert modulatory effects on dopaminergic neurons of the substantia nigra. The existence of a nigrohypothalamic pathway is also indicated by the results of Kizer et al. [1976], who found that lesions in the zona compacta of rat substantia nigra results in decreased DA levels in the median eminence.

PHYSIOLOGICAL SIGNIFICANCE OF MANGANESE IN BRAIN

Free-radical Scavenging

Although the brain, by virtue of its ingredients (high oxygen and lipid status, elevated metals), is an ideal milieu for the generation of free radicals and is susceptible to the neurodegenerative effects of lipid peroxidation, only a paucity of data is available concerning antioxidant vectors available

to deal with potential free-radical pathology. Superoxide dismutase is present as the Cu-Zn-cytoplasmic free-radical scavenger of O_2^-, as is the manganese form of this enzyme which is localized in the mitochondria. However, no convincing studies on their regional distribution in human or animal brain have been reported. The most effective of enzymes decomposing H_2O_2, catalase, is almost absent in CNS tissue. Glutathione peroxidase, which also scavenges H_2O_2, is present in brain but in low yelds [DeMarchena et al., 1974]. It is also dependent on glutathione availability for expression of its activity. Catalase can act as a "safety valve" when excessive H_2O_2 is present and prevent cell damage by this radical [Chance et al., 1979]. It is thus apparent that under conditions in the CNS that lead to the production of large amounts of H_2O_2 there is an apparent weakness in both amount and reactivity of the available inactivating enzymes. Thus, excess H_2O_2 production in the brain could present a serious threat to neuronal integrity and function, since it is a source for production of violently reacting oxidants such as ·OH and singlet oxygen.

At this point, a brief review of some of the devices used by the brain for dealing with toxic species of oxygen as well as the putative role of manganese in these processes may be pertinent.

Ascorbate

Although ascorbic acid is a powerful antioxidant and is present in very high concentration in brain, the reduction of the vitamin to dehydroascorbate results in excessive amounts of both H_2O_2 as well as O_2^-. It has also been demonstrated to potentiate the cytotoxicity of 6-OHDA. Ascorbate can undergo autoxidation, especially in the presence of copper ions, and is located in considerable amounts in rat brain hypothalamus [Subramanian, 1977]. The reasons for the stability of ascorbate in its physiological milieu are unknown, but endogenous inhibition to prevent autoxidation of the vitamin would play a crucial role. Ascorbate injected by various routes in rats has been shown to result in behavioral and neurotransmitter alterations [Tolbert et al., 1979]. Although ascorbate is an absolute cofactor for dopamine-β-monooxygenase (E.C. 1:14:17:1), the enzyme is inactivated in the presence of ascorbate [Brown et al., 1978] and it is an enigma as to how dopamine-β-hydroxylase (DBH) can function in catecholamine storage vesicles of the adrenals, which contain extremely high concentrations of the vitamin. A similar situation prevails in rat brain hypothalamus where ascorbate content is especially elevated, and is further enriched during development [Subramanian, 1977]. Since the rate of inactivation of the enzyme is enhanced by Cu^{2+}, which catalyzes ascorbate autoxidation, it is generally thought that the inactivating agents are H_2O_2 along with other toxic by-products. The denaturation process is reversible by catalase addition [Brown et al., 1978]. In this regard, both the adrenals and rat brain hypothalamus contain the highest content of ascorbate (along with DBH),

and both tissues are also prominent in their affinity for manganese. Manganese may serve a protective role in such tissues by preventing enzymatic denaturation of DBH initiated by ascorbate autoxidation by-products. This would be accomplished owing to the metal ion's preferential reactivity with toxic oxygen and related by-products.

Glutathione peroxidase

Under *in vitro* conditions in a number of tissues, divalent manganese is an effective inhibitor of lipid peroxidation and is also able to exert considerable inhibitory efficacy in rat brain tissue under experimental conditions of neurointoxication (Fig. 1). The amount of manganese required under *in vitro* conditions to inhibit peroxidation is about 5 μM for 50% inhibition. This concentration is much lower than that found endogenously in rat brain tissue and is well within the physiological range encountered in CNS tissue of the rodent [Donaldson et al., 1974]. Considering the propensity of the CNS to elaborate cytotoxic lipohydroperoxides, only a paucity of data exist concerning the activity and regional distribution of the key scavenger,

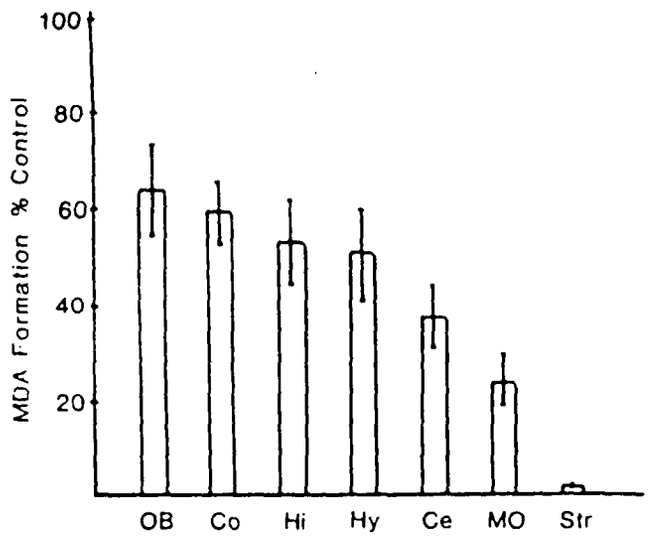

Fig. 1. Effect of manganese administration to neonatal rats on lipid peroxidative activity in brain regions. Duplicate homogenates of dissected rat brain in Tris buffer from manganese-treated and saline control neonatal rats were incubated for 2 h at 37°C in a shaking water bath and malonaldehyde formation estimated. Results ± SE represent the mean of ten samples each of control and manganese-treated animals. all regions from manganese-treated rats were highly significant ($P = > 0.001$). Abbreviations: OB, olfactory bulb; CO, cortex; Hi, hippocampus; Hy, hypothalamus; CE, cerebellum; MO, medulla oblongata; Str, striatum. [From Donaldson et al., 1982.]

glutathione peroxidase. DeMarchena et al. [1974] found very low activity of this enzyme in the brain of a number of species and concluded that brain tissue contains insufficient glutathione peroxidase activity to provide protection from peroxidative damage. Manganese may play an accessory role with glutathione peroxidase in critically sensitive areas of CNS in attenuating lipohydroperoxide proliferation.

The basal ganglia, especially the putamen, is selectively vulnerable for the initiation of free-radical-promoted lipid peroxidation since it contains the highest endogenous levels of dopamine and manganese in human brain. This combination, along with the high unsaturated fatty-acid content of nerve terminals in this region, and high oxidative status, renders it susceptible to damage from free radicals generated by catecholamine autoxidation. Selenium is a constituent of glutathione peroxidase, a scavenger of H_2O_2, and, like manganese, is concentrated in the putamen. In peripheral tissues the distribution of the enzyme reflects the endogenous selenium concentration, and the activity of this enzyme is drastically reduced under conditions of experimental selenium deficiency. Interestingly, selenium depletion studies have shown that there is a resultant decrease in antioxidant activity due presumably to reduced glutathione peroxidase activity, and this is accompanied by tissue damage arising from enhanced lipid peroxidation. In this connection it is of considerable interest that manganese and selenium, which are two powerful antioxidant metal ions, share a remarkable degree of correlation with each other in basal ganglia tissue (Fig. 2).

Superoxide dismutase

In relation to the inactivation of superoxide radicals in biological tissues by superoxide dismutase (SOD), it is a conundrum why both forms of the enzyme effecting detoxification (Cu-Zn and Mn) lead to a reaction product, H_2O_2, which is itself a powerful oxidant capable of considerable damage per se or, perhaps most especially, through its precursor status as a progenitor of the powerfully effective cytotoxin, the hydroxyl radical (·OH). This is an especial enigma in the CNS where the H_2O_2 scavenging enzyme, catalase, is present in only about 1/100th of the activity found in liver. In addition, cytoplasmic Cu-Zn-SOD is itself inactivated by H_2O_2 whereas the mitochondrial Mn-SOD is not affected. Such a differential effect by H_2O_2 on the two forms of SOD may confer a distinct advantage on cellular processes whose metabolism results in excessive generation of H_2O_2. Thus, the elevated content of manganese in rat brain hypothalamus may be indicative of a functional role for this metal ion. A factor lending credence to this concept is the recent demonstration that the highest catalase activity in rat brain is localized in the hypothalamus [Brannan et al., 1981]. It is in fact possible to envisage two such roles for manganese: (1) as a participant in the prosthetic moiety of superoxide dismutase where its ability to scavenge superoxy radicals would, unlike Cu-Zn-SOD, be unaffected in regions of high H_2O_2

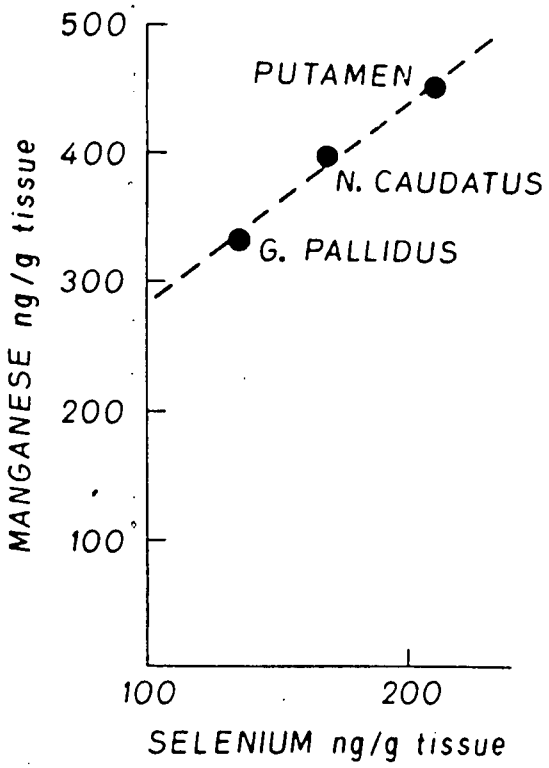

Fig. 2. Correlation of manganese and selenium in the basal ganglia regions of human brain. [Figures computed from data of Larsen et al., 1981.]

activity; (2) the neuron is particularly well endowed with pyrophosphates present as nucleoside triphosphates which would lend stability of manganese in the trivalent, Mn^{3+}, configuration. When present as a manganese-pyrophosphate complex, manganese can bring about the decomposition of H_2O_2 and is itself reduced in the process. The ability of manganese to react with and bring about the decomposition of a toxic by-product like H_2O_2, in addition to its facility in scavenging superoxide radicals, could present an enormous advantage to cells of elevated oxidative status in addition to reinforcing other cellular processes involved in active oxygen detoxifying mechanisms. In this regard we have found that, of several complexes tested, the phosphate complex of manganese exhibits the greatest inhibition of rat brain lipid peroxidation (Table 3).

TABLE 3. Inhibitory Effectiveness of Various Manganese Complexes on Lipid Peroxidation in Rat Brain

	Percent of control (100)[1]		
Manganese	5 nM	10 nM	20 nM
Pyrophosphate	85	30	5
Lactate	80	25	20
Gluconate	80	45	18
Tartarate	70	25	15

[1]Lipid peroxidation in whole-brain homogenates was determined by thiobarbituric acid reaction [Donaldson et al., 1982].

Manganese Regulation of Redox Homeostasis

Elucidation of the mechanisms whereby manganese induces insult to nervous tissue requires an understanding of the function this cation plays under normal conditions. Examination of some of the physical properties of manganese indicates that this metal ion could play an effective role in controlling redox and free-radical reactions in cells. These properties include (1) Manganese has a unique ability to assume multioxidation states; (2) in the Mn^{2+} state it is an effective scavenger of superoxide anion (O_2^-); and (3) in the Mn^{3+} state it may effectively decompose H_2O_2.

Free radicals such as superoxide anion (O_2^-), the hydroxyl radical ($\cdot OH$), and the important precursor, H_2O_2, are highly reactive species intracellularly and possess potent neurotoxic ability. For example, the powerful neurotoxin 6-hydroxydopamine (6-OHDA) elicits its neurodegenerative effects by virtue of its ability to release some or all of the toxic triad of free radicals [Graham et al., 1978; Graham, 1984]. Paradoxically, free radicals may also drive certain enzyme reactions. "Controlled" or "endogenous" amounts of specific radicals can therefore fulfill a physiological function. A good example of this is the norephinephrine-synthesizing enzyme, dopamine-β-hydroxylase (DBH). Both norepinephrine (NE) and DBH are present in highest yield in the hypothalamus [Mackay et al., 1978]. DBH is a metalloenzyme containing copper as part of its prosthetic moiety. It also has an absolute cofactor requirement for ascorbate. Ascorbate reduction elicits O_2^- which reduces the Cu^{2+} to Cu^+ of DBH's copper moiety. The ability of O_2^- to react with Cu^{2+} provides the redox cycle necessary for enzymatic homeostasis. Accordingly, the cycling between cupric and cuprous forms of copper in DBH maintains the balance of redox reaction necessary for enzyme function and synthesis of NE. It is also apparent that conditions leading to depletion of O_2 within NE neurons would effectively halt synthesis of NE. In the divalent state, Mn^{2+} is an effective scavenger of O_2^-. Its particular function in the hypothalamus of rat brain may lie in controlling the amount of free

radicals generated both by ascorbate reduction (for operation of DBH) as well as from other mixed function oxidase systems present in this region. These include tyrosine hydroxylase, (TH), which also can utilize ascorbate, and monoamine oxidase. The latter deaminating enzyme is present in highest amount in hypothalamus and produces large amounts of H_2O_2 during its metabolism. Because of its ability to react with O_2^-, the elevated content of manganese in the hypothalamus may be explicable in that it subserves a role related to regulation of DBH activity. This could be accomplished by controlling the amounts of ascorbate-generated O_2^- necessary for reduction of Cu^{2+} within DBH. By neutralizing excessive amounts of O_2^-, manganese would maintain homeostasis of the intracellular redox state. Owing to its effect in maintaining a fine balance in the oxidation-reduction bioenergetics peculiar to this brain region, manganese would thus exert indirect control over neurotransmitter function, as outlined in Figure 3.

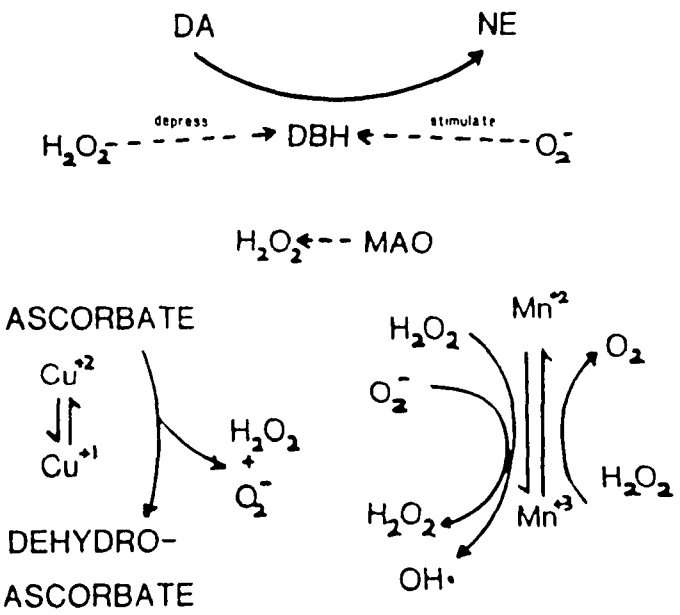

Fig. 3. Schematic illustration of the concept in which DBH activity in rat hypothalamus is regulated by free radicals arising from reduction of ascorbate. Hydrogen peroxide emanating from formation of the dehydroascorbate, along with H_2O_2 from other sources such as monoamine oxidase (MAO) may react with complexed manganese and undergo decomposition. Under conditions of neurointoxication by manganese, divalent ions would predominate and result in excessive scavenging of superoxide radicals required for DBH activity and other cellular processes. Manganese (Mn^{2+}) may also react with H_2O_2 to generate the profoundly cytotoxic hydroxyl radical.

In the divalent state Mn^{2+} acts as an effective scavenger of O_2^-.

$$2H^+ + 2O_2^- + 4Mn^{2+} = 4H_2O_2 + 4Mn^{3+} \quad (1)$$

Thus the inactivation of O_2^- is accompanied by generation of H_2O_2 and during the reaction Mn^{2+} is oxidized to the trivalent Mn^{3+} oxidation state. Decomposition of the resultant H_2O_2 arising from oxidation of Mn^{2+} as well as other sources of H_2O_2 within the cell could be accomplished by trivalent manganese:

$$4Mn^{3+} + 2H_2O_2 = 4Mn^{2+} + 4H^+ + 2O_2 \quad (2)$$

Divalent manganese is then recycled by reduction of the trivalent Mn^{3+}. In these reactions manganese serves a key function in bringing about the inactivation of two major species of free radicals, or their precursors, O_2^- and H_2O_2, owing to its unique ability to escalate and deescalate between the high and lower oxidation states with an appropriate free-radical "substrate."

The foregoing reactions are of course predicated on the assumption that hypothalamic manganese is present as a stable pyrophosphate complex. In this state the manganese-pyrophosphate complex has been shown to effectively bring about the disproportionation of O_2^- to H_2O_2 and O_2. However, manganese may well be present—perhaps entirely—as part of the prosthetic moiety of Mn-SOD. Since the regional distribution of Mn-SOD in rat brain is unknown, it is imprudent to tender an opinion as to what extent endogenously localized manganese is present as complexed pyrophosphate or bound within regulatory subunits of SOD.

Although enzymes are present in rat brain microperoxisomes, which can inactivate H_2O_2, their concentration and activity are negligible, and under conditions of excessive generation of H_2O_2 which prevail in rat hypothalamus they could not be considered effective primary scavenging agents. Thus, H_2O_2 inactivation by complexed manganese could provide an important detoxification device to high H_2O_2-generating compartments of the CNS.

The proposition that manganese may subserve such a detoxifying role in discrete CNS compartments as outlined here is supported by results of recent investigations demonstrating that lactic acid bacteria containing high intracellular levels of Mn^{2+} in a dialyzable form were devoid of true SOD [Archibald and Fridovich, 1981]. Conversely, those bacteria that possessed SOD did not contain high levels of Mn^{2+}. Manganese, present as Mn^{2+}, was able to scavenge superoxide radicals while exerting protection against both direct and indirect effects of this radical. The ability of high internal Mn^{2+} to provide microorganisms with an important defense against endogenous superoxide radicals and to exert such activity without partici-

pation in a metalloenzyme is important since elevated endogenous metal ion localization in a tissue has traditionally been viewed as merely indicating participation in a prosthetic group of a metalloenzyme. Since the possession of elevated manganese by lactobacilli in the study cited [Archibald and Fridovich, 1981] was found not to be primordial to true SOD, it is conceivable that manganese present in brain regions with especial vulnerability to free-radical pathology may constitute a strategic and unique device in the CNS for counteracting the damaging effects of oxygen-derived free radicals.

Exogenously administered manganese localizes in the hypothalamus [Donaldson et al., 1974; Bonilla, 1980; Deskin et al., 1981]. During neurointoxication by this metal ion, a principal effect of the accruing manganese would be to disrupt the normal cycle of redox control exerted by *endogenously located* manganese ion. Because of the affinity of Mn^{2+} for O_2^-, this would result in intensive scavenging of this radical by exogenous Mn^{2+}. Such accelerated scavenging activity by Mn^{2+} would result in depletion of "normal" amounts of O_2^- which arise from ascorbate reduction and are used to drive DBH activity. A principal effect of such O_2^- depletion would be to halt NE synthesis, resulting in a fall in endogenous NE level. Arrest of DBH synthesis would also inhibit further generation of O_2^- derived from ascorbate since there would be no further need for this DBH cofactor in NE synthesis. In this regard, we have recently found a significant decrease of NE in the hypothalamus of manganese-treated rats [Donaldson et al., 1982]. Thus the overall effects of excess manganese in the hypothalamus would be the following. (1) The finely tuned oxidation-reduction balance between lower- and higher-valency states of manganese would be upset since the oxidation state of the incoming manganese would be predominantly in the lower-valency Mn^{2+} form. (2) Hydrogen peroxide decomposition would be affected since this process is accomplished by higher-valency Mn^{3+}. The amount of Mn^{3+} available for inactivating H_2O_2 arising from oxidase reactions (tyrosine hydroxylase, monoamine oxidase) would be drastically decreased owing to lack of available O_2^- for generation of the trivalent manganese species. (3) Although exogenous manganese would actively scavenge cellular O_2^-, complete depletion of this anion would result in impairment of neurotransmitter (NE) function. On the other hand, lack of Mn^{3+} would prevent the decomposition of H_2O_2. Excess of H_2O_2 represents an important source for generation of the highly cytoxic hydroxyl radical, $\cdot OH$, which may be formed by reaction of divalent manganese and H_2O_2:

$$Mn^{2+} + H_2O_2 = \cdot OH + OH^- + MN^{3+} \qquad (3)$$

The foregoing reactions and the role of manganese are summarised in Figure 3. In view of the avidity of manganese for oxygen, it should be realized that the metal in excess could theoretically be capable of inducing a highly localized neuronal hypoxia.

FREE RADICALS IN NEURODEGENERATIVE DISORDERS

Though long considered an innocuous repository of unhydrolyzable cellular debris, and viewed by both neuropathologist and neurochemist for decades as a mere anatomical curiosity of cursory academic interest, recent advances in both neurohistochemistry and free-radical pathology indicate that the lipofuscin granule may play a role in the pathogenesis of neurodegenerative disease. For example, lipofuscin has long been recognized as an age pigment since its rate of accumulation in nervous tissue is proportional to the chronological age of the brain at autopsy, and its presence is taken as an indication of the sum total of preceding processes of lipid peroxidation. Since the process of lipid peroxidation is initiated by free radicals, measurement of lipid peroxides by their ability to react with thiobarbituric acid is a measure also of the extent of free-radical activity. To gain an appreciation of the possibility that lipid peroxidation may play an important role in the basic defect of the neurochemistry of degenerative diseases, it is of necessity particularly relevant to understand how this process arises in the CNS, and perhaps more especially, the neuronal mechanisms for counteracting free-radical toxicity. The major sites of lipid peroxidation damage within the cell are at biomembranes, especially those of subcellular organelles. Mitochondrial and microsomal membranes contain relatively large amounts of polyunsaturated fatty acids. These fatty acids include those with two, four, five, and six double bonds, for which the relative rates of peroxidation both *in vivo* and *in vitro* are one, four, six, and eight, respectively. In close molecular proximity to these polyunsaturated lipids of the membrane are some of the most effective catalysts for initiation of lipid peroxidation: trace metals such as iron, manganese, copper. During lipid peroxidation, semistable peroxides from free-radical intermediates are produced by direct reaction of oxygen with unsaturated lipid. Branching reactions of autocatalysis result from homolytic scissions of hydroperoxides, which can be caused by metal catalysts, particularly copper, iron, and manganese. Free-radical species produced during lipid peroxidation are chemically similar to the damaging radicals produced during radiation. In fact, much of our present knowledge of free-radical pathology has emerged from studies of radiobiology, and damage of membranous cell structures by lipid peroxidation is a signal feature of deteriorative mechanisms of cellular aging. A classic, albeit tragic, example of lipofuscin accumulation as a pathological entity is the extensive neuronal ceroidlipofuscinosis studies, particularly in juvenile amaurotic idiocy (Batten disease). Blindness, severe disablement, and progressive intellectual impairment are features of this literally morbid disorder.

A potentially important source of free radicals in the CNS, especially in the basal ganglia because of its high oxidative capacity, resides in the electron transport chain. However, these reactions are well controlled, and the chain is tightly coupled by virtue of the fact that many of the enzymes

and carriers are membrane-bound and in close juxtaposition to each other. Consequently, the lack of mobility induced by membrane binding and the ease of electron flow to a proximate carrier assure that an orderly series of radical reactions are confined within intact mitochondria and endoplasmic reticulum.

Catecholamine Toxicity

A major source of free-radical production in nervous tissue may arise from the high content of catecholamines present in that tissue. Graham and co-workers [1978] have found a direct correlation between the capacity of polyphenols for autoxidation and their cytotoxicity toward neuroblastoma cells in culture. The highest rate of autoxidation was found for 6-hydroxydopamine (6-OHDA), a potent neurotoxin, whose mode of action is generally accepted to arise through its capacity to liberate toxic oxidation by-products. These workers found evidence for two clearly separate modes of toxicity by the polyphenols tested: (1) the production of H_2O_2 and free-radical species O_2^- and $\cdot OH$ as a result of autoxidation, and (2) the production of quinone compounds which kill cells through inhibition of sulfhydryl enzymes and reactions with other nucleophilic groups within the cell. 6-Hydroxydopamine toxicity was believed to arise through the former mechanism. Dopamine also exhibited a high rate of autoxidation with concomitant neurotoxicity believed mediated by both mechanisms (1) and (2). Current investigation of the biochemical basis of manganism indicates that endogenous trace metals can influence lipid peroxidative processes, and this ability may have definite implications in relation to understanding the basic neurochemical dysfunction in neurodegenerative disease. For example, Mn^{++} in laboratory animals induces behavioral and neurochemical changes strikingly similar to those induced by 6-hydroxydopamine. This effect is not, as first considered, due to the ability of Mn^{++} to metabolize an endogenous 6-hydroxydopamine-like agent, but is likely due to the ability of the divalent ion to exert a similar mode of action as that of 6-hydroxydopamine itself. Such similarities between apparently diverse agents we believe is due to the ability of Mn^{++} to considerably enhance the autoxidation of dopamine with concomitant production of free radicals and toxic orthoquinone species.

Of particular importance relating to Mn^{++} ability to induce nervous tissue insult under *in vivo* conditions is that Mn^{++} can undergo oxidation with superoxide anion (O_2^-) to the trivalent form, Mn^{+++}. In the trivalent state manganese's ability to augment catecholamine oxidation is considerably potentiated. Since dopamine is the principal substrate for neuromelanin synthesis in the substantia nigra [Das et al., 1978], the depigmentation of this region, as well as the associated parkinsonian symptoms that accompany manganese intoxication, may be due to the ability of Mn^{++} to enhance considerably the synthesis of melanin with the simultaneous production of cytotoxic neuromelanin precursors [Graham, 1978, 1984]. Degeneration of

basal ganglia tissue could ensue owing to the toxicity of the free radicals induced or by their subsequent participation in lipid peroxidation. Whether or not these events actually occur under *in vivo* conditions in the central nervous system is not known, but on the basis of an abundance of *in vitro* evidence concerning free-radical involvement in lipid peroxidative reactions, the ingredients present in CNS tissue represent the ideal milieu for such a process to occur and to contribute substantially toward neurodegeneration. These are (1) high content of easily autoxidizable substrates, (2) high content of unsaturated fatty acids, and (3), in discrete brain regions, a high content of metals of strongly positive redox potential, principally manganese, and iron.

Basal Ganglia Degeneration and Manganese

In common with other neurodegenerative disorders extensive lipofuscin accumulation is evident in the putamen of Huntington diseased brains. A major clue toward understanding the neostriatal degeneration in this disorder may be generated from perusal of data acquired during investigation of some variant extrapyramidal disorders exhibiting similarities to Parkinson disease. In manganese intoxication, for example, mild to moderate degeneration of the substantia nigra is noted, but, like Huntington disease (HD), major changes in pathology are encountered in the neostriatum. Despite the paucity of data on the pathology of manganism in relation to CNS changes, earlier studies by Canavan et al. [1934] on one case revealed that caudate and putaminal damage was present. These workers considered that this case was representative of striatal-pallidal disease. Of particular relevance was their observation that the vascular bed as well as cells of the caudate nucleus contained an abundance of "black pigments." Another variant of an extrapyramidal disorder resembling both HD and parkinsonism is striatonigral degeneration. Putaminal dystrophy is a major force of this condition, which is frequently diagnosed as Parkinson disease owing to the clinical signs of akinesia, rigidity, and tremor prevalent in this rare disorder. Borit et al. [1975] have reported three cases of striatonigral degeneration (SND) in which only mild to patchy degeneration of the substantia nigra was observed. Others, however, find considerable loss of melanin in the substantia nigra but considerable pigment deposition in the putamen [Adams et al., 1964]. Of considerable interest was the finding [Borit et al., 1975] of excessive pigment deposits in the putamen later identified as lipofuscin and neuromelanin. Such pigmentation is exclusive to the putamen and is not found in other nuclei. Neuromelanin is not normally found in this region, and its location there in this basal ganglia disorder indicates that SND and manganism share similar features of their neuropathology. It is highly likely that the "black pigment" found by Canavan et al. [1934] may be in fact neuromelanin. Borit et al. [1975] found that manganese levels in basal ganglia tissue from two of three of their cases of SND were

markedly elevated. The putamen contains the highest content of dopamine as well as the highest manganese concentration. Accordingly, an attractive speculation is that the pigmentation excess observed in these two extrapyramidal disease variants, manganism and SND, may arise from enhanced autoxidation of endogenous dopamine stores, or, alternatively, from its accelerated synthesis in the substantia nigra as a result of nigral overactivity. In this connection, it is significant that at autopsy the substantia nigra of patients given extremely large amounts of dopa is marked by an accumulation of neuromelanin which is located extraneuronally [Campbell et al., 1967].

Apart from the putaminal atrophy common to both SND and HD, a number of other features are shared. For example, the large cells of the caudate and putamen are relatively spared in both SND and HD, whereas there is a disproportionate degeneration of small nerve cells in both syndromes [Adams et al., 1964]. Also, in some cases of SND, mental deterioration is observed [Adams et al., 1964]. This feature of psychic disturbance is interesting, since in manganese intoxication the extrapyramidal manifestations of this condition are preceded by a psychiatric phase.

Manganese and Phenothiazine Neurotoxicity

Chlorpromazine (CPZ), used extensively in the treatment of schizophrenia, can undergo ring hydroxylation to form mono- and dihydroxylated derivatives. One of these derivatives, 7-8-dihydroxychlorpromazine (7-8-OHCPZ), is found in the urine of schizophrenic patients solely on CPZ therapy and is a highly reactive metabolite. Earlier work by Cotzias et al. [1964] has noted that the production of experimental extrapyramidal symptoms in animals occurs only in those species close to man which possess melanin in the substantia nigra, usually primates. Cotzias and Borg [1962] have also noted that the ability of phenothiazine derivatives to produce extrapyramidal symptoms parallels their ability to bind to melanin.

Salazar et al. [1978] have shown that [^{3}H]-chlorpromazine binds strongly to isolated neuromelanin granules from the substantia nigra, and they have suggested that chronic phenothiazine administration could result in destruction of this brain region. Bird et al. [1969] demonstrated also that chronic phenothiazine administration in monkeys led to lipofuscinosis along with elevation of manganese levels in the putamen. Examination of the autopsy reports of patients following prolonged phenothiazine administration reveals a similarity to the neuropathology of parkinsonism and also of that seen in aging brain. A major finding is that of extensive lipofuscinosis or the "aged-brain syndrome." Christensen et al. [1970] noted that depigmentation of the substantia nigra occurred in 27 out of 28 brains in patients who had received chlorpromazine up to 3½ years. More recently, Jellinger [1977] found lesions in the caudate nucleus in two patients who had received phenothiazines for 11 years and who had exhibited pronounced extrapyr-

amidal symptoms. Such effects, especially in the basal ganglia, could reflect the affinity of chlorpromazine to bind to brain melanin. The ability of chlorpromazine to induce neurotoxicity particularly in the basal ganglia may be related to its ability to undergo autoxidation to the semiquinone derivative. Borg and Cotzias [1962a,b] examined the ability of a number of metal ions to catalyze chlorpromazine autoxidation with production of the semiquinone and found that the most effective metal was manganese. They suggested that the mechanism of action of the phenothiazine *in vivo* may be related to its unique ability to form coordination complexes with this cation and that this reaction was enhanced when manganese was present in the trivalent state.

It would appear therefore that the ability of chlorpromazine to induce Parkinson-like syndromes in man could be related to its unique ability to undergo free-radical formation in the presence of a metal ion, manganese, and thus the similarity of effects produced by both manganese neurointoxication and phenothiazine become explicable in terms of a site of action which is common to both agents, the neuromelanin-containing regions of brain. It is known that the degree of dopamine depletion in the striatum parallels the degree of destruction of the melanin cells within the substantia nigra, and these effects are seen in advanced aging, manganism, and phenothiazine intoxication as well as in Parkinson disease. Graham [1978] considers that "in idiopathic parkinsonism degeneration of the cells in the substantia nigra is probably due to toxic intermediates formed during neuromelanin synthesis . . . ": this premise would seem to be born out by the clinicopathologic data observed in the chemically mediated forms of parkinsonism.

Manganese and Cerebral Senescence

Manganese-dependent superoxide dismutase is a mitochondrial-bound enzyme, and an intriguing finding is that the enzyme can be induced by exposing animals to a high oxygen content. Recently Vanella et al. [1982] demonstrated that exposing young rats to a high oxygen atmosphere resulted in enhancement of brain Mn-SOD whereas Cu-Zn-SOD activity was unchanged. However, in old rats Mn-SOD levels were not induced by oxygen exposure, indicating that Mn-SOD in mitochondria may play a critical role in the neurochemical events involved in cerebral senescence. Mavelli et al. [1982] also found that although both Cu-Zn-SOD and Mn-SOD increase progressively with age in the rodent CNS, Mn-SOD increases to a greater extent. Such findings help explain the ability of chronically administered manganese to protect important mitochondrial rat brain enzymes such as NAD-linked isocitrate dehydrogenase and glutamic acid decarboxylase from the usual decrease in activity that is a normal corollary of the aging process [Lai et al., 1981].

Since our studies [Donaldson et al., 1982] have shown that short-term manganese injections in neonatal rats result in a dramatic decrease in lipid peroxidation in brain compartments, especially in areas of high oxidative status like the basal ganglia, it is conceivable that the protective effects of manganese on critical brain enzymes affected by the natural deteriorative process of senescence [Lai et al., 1981] may be due to its facility in scavenging or curbing proliferation of toxic species of oxygen which would otherwise impair enzymatic and related critical processes of cellular activity. Harman [1982] has suggested that the maximum life-span of a particular species is linked directly to the rate of oxygen consumption. Since free-radical elaboration is intimately related to respiration, it is suggested that the mitochondria represent the molecular clock of the cell. An accruing body of evidence indicates that the continuous injury by toxic oxygen species throughout the life-span of a cell plays a decisive role in dictating longevity. Toxicity arising from free-radical species of oxygen generated in mitochondrial respiration and leaking from membranes may be an important source of endogenous production of potential cytotoxins in animal tissues. The age pigment lipofuscin is always predominant in aging brain preparations, and this pigment is thought to result from oxygen-dependent destruction of biomembranes [Miquel et al., 1980].

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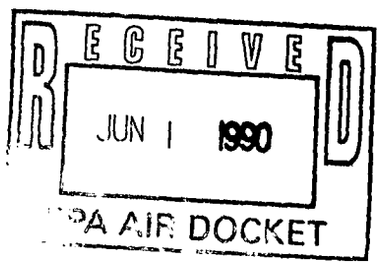

Dr. Joan M. Cranmer, Editor-in-Chief
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Dear Dr. Cranmer:

I am enclosing three copies of a manuscript entitled "Manganese, a Physiological Marker for Criminal Violence" for possible publication in NeuroToxicology.

Sincerely yours,

Louis A. Gottschalk

Louis A. Gottschalk, M.D., Ph.D.
Professor of Psychiatry

A B S T R A C T

MANGANESE, A PHYSIOLOGICAL MARKER FOR CRIMINAL VIOLENCE

(Gottschalk et al.)

There are long-standing viewpoints that impulsive and violent behavior may stem from brain dysfunction or damage secondary to head injury, disease, or toxic chemical substances. This research has aimed to examine the relationship between potentially toxic metals and aberrant behavior, especially violent activity, through the nonintrusive technique of hair analysis for trace elements. In an initial study (Phase I) it was not possible to replicate findings of others who reported high lead, cadmium, and copper in violent offenders. However, high levels of manganese were found in prison versus control groups. In Phase II, the possibility of artifactual results arising from prison cooking utensils was controlled for by sampling early after incarceration. Phase III was included to substantiate the initial post hoc findings in an additional jail population. In both phases, significantly elevated manganese levels were found in the hair of violent versus nonviolent subjects ($P<0.0001$). A review of the effects of manganese at deficient and toxic levels does not provide a simple answer as to why manganese levels are elevated in the hair of individuals who have been incarcerated for violent behavior. Our study does not implicate the prison environment or soaps and shampoos used in California prisons. A cofactor such as alcohol, dietary deficiencies, or psychosocial factors, might act in combination with mild manganese toxicity to precipitate violent behavior.

MANGANESE, A PHYSIOLOGICAL MARKER FOR CRIMINAL VIOLENCE

LOUIS A. GOTTSCHALK*, TESSIO REBELLO**, MONTE S. BUCHSBAUM*, HOWARD G. TUCKER, E. L. "RED" HODGES

In animal models impulsivity and violent behavior have been reported to be the result of brain dysfunction or damage, especially in the cortical and limbic areas (May and Ervin, 1970). Several human studies indicate that some violent offenders have histories of head injuries, learning disabilities or neurological problems (Elliott, 1984; Pincus, 1980; Lewis, et al., 1980). This observation has been corroborated by neuropsychological testing which reveals evidence of brain function impairment in a small proportion of offenders (Kelly, 1982; Mungas, 1988). The mechanism whereby some brain lesions contribute to violent behavior, however, is not well understood (Fishbein and Tatcher, 1986). Toxic metals such as lead are correlated with brain lesions and/or dysfunction in humans and rats (Chandra et al., 1970; Needleman, 1979; Schauss, 1981). Elevated tissue and dentine levels of toxic metals have been shown to correlate with learning disability and intellectual impairment (Needleman et al., 1979; Thatcher et al., 1982). A significant relationship between intellectual impairment, learning disability, delinquency, and criminality has not been

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firmly established. Some authors assert that learning disability may predispose an individual to emotional outbursts, and disruptive behavior (Poremba, 1975; Slavin, 1975).

Hair has the potential to become a toxic element diagnostic tool. It is collected without trauma to the individual, and can be analyzed relatively easily. Trace elements are accumulated in hair at concentrations that are generally higher than those present in blood or urine. Minerals once situated in the hair are no longer in dynamic equilibrium with the body since hair is a metabolic end product (Klevay, 1978; Katz 1979). Hair, thus, may provide a record over time of past toxic minerals status since hair grows at the rate of approximately 1.5cm per month (Schroeder and Nason, 1969; Maugh 1978). The pathogenesis of violence possibly can be traced through hair analysis.

A number of concerns have been raised about the utility of hair as a diagnostic tool. These mainly center around the collection, preparation, analysis of hair samples, interpretation of data, and hair that is contaminated by perm, dye or bleaching, since this is always a major concern. In this study we controlled for such past shortcomings.

METHODS AND PROCEDURES

The study reported here was performed in three stages. The first was undertaken in collaboration with California State University at Stanislaus and the Health Research Institute in Chicago, Illinois to evaluate the overall pattern of concentration of twenty three minerals in hair in a group of

violent criminals. Particular attention was paid to lead, cadmium, and copper levels, since these had been previously reported to be associated with violent behavior (Schauss, 1981). The object of this first phase was to attempt to replicate this report with California subjects. In the subsequent two phases, hair samples were collected within an average of 20 days of incarceration of subjects in order to control for the possible contamination of the subjects' hair by chemical substances from the prison metal cooking utensils. In Phase II and Phase III the generalizability of a manganese/violence correlation discovered in Phase I was examined.

SUBJECTS

PHASE I

In 1984, during the first phase, one hundred and four male offenders were randomly selected from a class III prison for convicted felons (Deuel Vocational Institute, Stanislaus, California). The Experimental group of 39 Caucasian, 33 Hispanics, and 32 Blacks (n=104) was incarcerated for no less than one year prior to the hair sampling. They had been convicted of felonies, the majority of which were murder, rape, armed robbery, and assault with a deadly weapon. The mean age of all participants was 29 years (range 19-65 years). Information was obtained on the most recent offense, chronicity of criminal offense and history of each participant. A town control group of 52 controls, who reported never having been convicted of a

felony, was recruited from barber shops in the Tracy/Stanislaus area, within a 50 mile radius of the penal facility. Thirty one prison guards, from the Deuel facility, were also selected as in-house controls for this study, ("Guard Control" group). The subjects were matched for age, sex, and race (Controls n=83) (See Table 1).

PHASE II

In the second study, which took place in 1987, sixty inmates were recruited (mean age 27 years, range 18-47 years) who were awaiting trial on charges of violent crimes, (murder, rape, assault with a deadly weapon), from the Los Angeles and San Bernardino County jails. The group, which consisted of 20 Caucasians, 20 Hispanics, and 20 Blacks, had been in custody fewer than 11 days (Los Angeles) and three months (San Bernardino) (mean=20 days). Forty two conviction-free subjects who reported never having committed a violent act on another human served as controls. They were recruited from the surrounding area barber shops and matched for age, sex, and race.

PHASE III

The third phase of the study was undertaken in 1988 at the San Bernardino County Jail. Twenty nine male Caucasians who were charged with a violent crime were recruited. Their ages ranged from 18 to 39 years, with a mean of 27 years. The control group of 59 noncriminal male Caucasian subjects had a mean age of 33 years (range 16-49 years). Thirty of the control subjects were

recruited from the San Bernardino County area while 29 came from the Orange County area located 50 miles away. The same criteria for violence were applied as in prior phases.

METHODS

Approximately one-half gram samples of hair were taken with stainless steel scissors from the nape of the head and within 7 cm. of the scalp. Subjects who had used dyes, bleaches or other hair chemical treatments were excluded from the study. Each subject was paid $10 for his participation.

The assay of the metals in the hair samples was performed by Doctors Data Laboratories, Inc. of West Chicago, Illinois. Hair samples were washed successively in detergent (Triton X-100), acetone and deionized water. The samples were then oven-dried, weighed, and solubilized in a mixture of perchloric and nitric acids. The solutions were analyzed by inductively coupled plasma atomic emission spectrophotometry for the following elements: calcium, magnesium, sodium, potassium, copper, zinc, iron, manganese, chromium, cobalt, lithium, molybdenum, phosphorous, selenium, silicon, vanadium, lead, cadmium, arsenic, mercury, aluminum, nickel and berylium. A standard hair sample was randomly added to each group of approximately 25 samples to test for reliability in Phase I and II.

In the first study, hair samples were assigned a code number which blinded the investigators from identifying sample origin. An independent party, a retired Superior Court Judge, held the code while the samples were being analyzed. Only after the data

were tabulated were the codes broken. The sources of he hair samples in Phase I and Phase II were also blinded.

RESULTS

Phase I was undertaken primarily to replicate research in which high lead, cadmium, and copper had been found in violent offenders as reported by Schauss (1981).

In Phase I it was observed that the elements of interest were not normally distributed among the two populations. Two approaches were used to test for statistical significance. Data were either tested nonparametrically by the Wilcoxon rank-sum test for tied midranks or by the Kruskal-Wallis test (Lehmann, 1975) or tested parametrically (Student "t" and ANOVA test) after log transformation of the data. Data are presented as mean $\pm$ SE.

PHASE I

In this first phase, at the Deuel Vocational Institute, the mean level of lead in the hair of prisoners was 12.1 $\pm$ 5.19ppm (range = 1-535); whereas that of the town controls was 7.3 $\pm$ 1.01 (range = 1-39 ppm). Mean lead levels in prison guards was 13.3 $\pm$ 6.55 (range = 1-208). Mean cadmium level was 0.48 $\pm$ 0.062 (range = 0.1-3.2 ppm) for the prison group, 0.74 $\pm$ 0.161 ppm (range = 0.1-7.7) for the guards, and 0.71 $\pm$ 0.169 ppm (range = 0.1-5.1) for the town control group. The differences were not statistically significant. The other metals of interest, e.g., copper, also showed no statistically significant differences between the groups.

Though Schauss' (1981) report of an abnormal hair metal pattern in violent individuals could not be corroborated, relatively large differences were found in the level of manganese between the prisoner and control groups. The prisoners had a mean level of 2.20 ± 0.24 ppm (range = 0.10-17.46). The town controls had a level of 0.30 ± 0.043 ppm (range = 0.07-1.89). The guard controls had a mean level of 0.55 ± 0.128 ppm (range = 0.08-3.58). The differences between the prisoners and the two control groups were statistically significant. The one way ANOVA test statistic was 20.886, df=2, with a P-value indistinguishable from zero in both cases. The Kruskal-Wallis test statistic was 79.87, df=2, with a P-value indistinguishable from zero.

PHASE II

The Los Angeles/San Bernardino study re-examined the levels of metals previously reported. It focused on manganese. Again, the only unusual metal pattern was significant differences in manganese levels. The jail group possessed a mean manganese level of 1.39 ± 0.297 ppm (range = 0.08-3.41) while the controls had a mean level of 0.41 ± 0.070 ppm (range = 0.08-1.61). The difference was statistically significant with the Wilcoxon 2 sample test statistic 3608.5 and P-value indistinguishable from zero. The difference was also significant for the t-test with value of t-statistic of 5.18; at df=98, the P-value was also indistinguishable from zero.

PHASE III

The third phase replicated the above findings on Caucasian subjects only, revealed a mean manganese level of 0.71 ± 0.144 ppm (range = 0.16-3.55) in the jail group and 0.33 ± 0.033 ppm, (range = 0.07-1.52) for the control group. The difference was statistically significant. Using the Wilcoxon test, the statistic of 1679.5 had a P<.001. The t test statistic of 4.006 with df=86, yielded P<.001.

When the three studies are grouped together, we observe that the mean manganese level in the hair of 197 prisoners/jail inmates was 1.62 ± 0.173 ppm (mean = 0.96 ppm; range = 0.08-17.46 ppm). The controls (n=184) had a mean level of 0.35 ± 0.020 ppm (mean = 0.23 ppm; range = 0.07-1.88 ppm). The differences among the three groups (prisoners, guards, controls) were statistically significant using both the Kruskal-Wallis test (test statistic 123.272, df=2, P-value indistinguishable from zero); and ANOVA (test statistic 30.559, df=2, P-value indistinguishable from zero).

It was empirically observed that if a value of 0.7 ppm and above was used as a discriminant function, then 124 out of a total of 191 (62%) prisoners could be selected. In contrast only 19 out of 184 controls (10.33%) were selected. In all three phases the Irwin-Fisher exact test, which compares the marked-to-total ratios of prisoners versus controls, was found to have P-values of 0, .0004 and .0018 respectively.

The figure shows manganese levels in controls and prisoners grouped according to the facility in which they were incarcerated or where they lived. All the prison/jail groups had a mean

manganese value above 0.7 ppm; whereas the mean manganese levels in all the control groups were below this value. According to Doctors Data, 0.7 ppm occurs one standard deviation out on the curve of a normal population for Caucasian males.

DISCUSSION

Abnormal trace mineral metabolism may be a factor contributing to criminal behavior, and careful hair trace metal analysis is a tool for studying the underlying biochemical basis of criminal behavior. Schauss (1981) and Cromwell et al., (1989) have observed that hair from violent individuals may possess a metal pattern which can distinguish them from non-violent individuals. These workers have found abnormalities in about a dozen or so major electrolytes and trace metals. Curiously, the metal "pattern" in the two studies, which were performed at different locations, were not alike, suggesting perhaps, a geographical influence. Nevertheless, it is difficult to comprehend how such an unrelated array of elements could influence behavior, since some of the elements (lead, cadmium, sodium, silicon, potassium, copper, cobalt, etc.) are metabolically dissimilar and functionally different.

In the present study, the consistent finding was a significantly elevated level of manganese in hair of persons either charged with, or imprisoned for, a violent crime. Curiously, Schauss (1981) did not find elevated manganese in his violent offenders (N=31, 0.56 ± 0.64ppm) as compared to his controls (N=31, 0.74 ± 0.41ppm).

Although manganese can modulate brain chemistry, the mechanism of its influence on behavior is unknown. Manganese toxicity probably results from its action in aiding the depletion of dopamine, a neuro-transmitter (Donaldson et al, 1982).

Manganese is an essential trace metal. It can act as a cofactor in a number of enzymes, such as hydrolases, kinases, decarboxylases, and transferases. It is an important component of superoxide dismutase, a free-radical scavenging enzyme. Human requirements for manganese are not well defined. However, gross deficiencies have been reported to produce weight loss, dermatitis, nausea and slow growth of hair (Doisy, 1973). Lower manganese levels in blood and hair have been reported to occur in epileptics compared to normal controls (Papavasiliou et al., 1979). Toxic reactions to manganese on the other hand have been reported only in some individuals exposed to very high concentrations of manganese ores (Cotzias, 1958; Cawte, 1984). The first symptoms of manganese intoxication are vague, but they can appear suddenly and progress rapidly. Barbeau et al. (1976, p.339) have reported that extreme fatigue, somnolence, and irritability usually precede behavioral manifestations, the so-called manganese madness ("locura manganica"), which includes violent behavior, and according to Penalver (1955), involvement in "stupid" crimes. Compulsive acts, emotional instability ("easy laughter, or crying"), and hallucinations are other common presenting symptoms (Cotzias, 1958). These authors have observed that neurological signs appear towards the end of the first month of clinically evident disease in the form of generalized muscular

weakness, followed by difficulty in walking, headaches, impaired equilibrium and speech. Elevated manganese levels have also been found to be associated with dementia and extrapyramidal signs (Banta and Markesbery, 1977). The manganese levels found in the hair samples of our prisoners were lower than the levels found in persons known to have toxic exposure to manganese.

Recently, manganese toxicity has been described in aboriginal inhabitants of Groote Eylandt, an island located in the Northern Territory, Australia (Cawte, 1984; Killburn, 1987). Affected natives display a variety of clinical symptoms which include motor neuron disease, connective tissue disorders, congenital malformations, and psychiatric excitement. The record of arrests and incarcerations of the native population on Groote Eylandt is said to be the highest in Australia (Donaldson, 1988). According to Cawte and Florence (1989), "Groote Eylandt ecology shows a synergistic chemistry which may aggravate the effects of manganese". A low level of calcium in the diet is harmful, in that manganese can displace calcium from nerve endings. This is more likely to happen in individuals with high manganese and low calcium levels. Zinc deficiency can also facilitate manganese toxicity. Zinc status in Aborigines is generally low, especially in cases of alcohol abuse, frequently associated with low zinc and magnesium status". More information is needed to determine what individual factors contribute to the ease with which manganese is absorbed from the environment.

The biological half-life of manganese is relatively short (Mahoney, 1968). Therefore, monitoring manganese at the low

levels found in blood and urine only reflect relatively recent exposure. The most convenient and accessible record of manganese exposure, over longer periods, appears to be the manganese concentration in hair (Stauber et al., 1987). However, the assay of manganese in the hair of unexposed individuals has revealed considerable variability, depending on the assay method used (Guillard et al., 1984). Using one standardized method, the adult geometric mean scalp hair manganese concentration was 0.64 ppm for males in 102 white male subjects in Metropolitan New York ranging in age from 16-51 (Creason et al., 1975). Eskimos showed higher hair values for manganese (Gordus, 1973). To minimize the relatively wide range of published values for the determination of manganese in hair, and thereby to maximize the analytical reliability of manganese hair levels, Guillard et al. (1984) recommend an assay method that was used in this study, namely, atomic emission spectrophotometry. The manganese levels observed in our study agree closely with those found by Guillard and his co-workers in a group of healthy French volunteers (0.26 $\pm$ 0.05 ppm).

It must be emphasized, however, that the relatively higher levels found in our prison/jail group in no way reflect clinically toxic metal burden. Typically, those who are exposed to high levels of manganese pollution possess hair values that are two to six times higher (Cawte and Florence, 1989).

Why manganeses levels are elevated in the hair of individuals who have been incarcerated for violent behavior is a question we cannot answer at this time. Such an abnormality, if

verified by further studies, might be directly or indirectly related to violent behavior. Our studies do not implicate the prison environment as a contributing factor to this elevated manganese, since our Phase II and III controlled for length of time incarcerated. It is known that increased levels of melanin in blacks are positively correlated with concentrations of heavy metals, e.g., manganese (Cawte and Florence, 1989; Pfeiffer and Mailloux, 1988). However, all our groups were matched for race. We did not observe a positive correlation between iron and manganese levels in hair, thus ruling out contamination from scissors during hair sampling. Furthermore, analysis of shampoos and soaps commonly used in California prisons revealed negligible amounts of manganese. Hence, the elevated manganese appears to have been present before imprisonment.

Although there is considerable evidence that manganese can act as a neurotoxin (Cotzias et al., 1971; Donaldson et al., 1982), our review of the literature does not point to convincing evidence that manganese "toxicity" directly leads to violence. Rather, manganese poisoning seems to be associated usually with extrapyramidal disorders, such as, a Parkinsonian syndrome. Perhaps a cofactor, such as, alcohol or other chemical substance abuse, or psychosocial factors, acting in concert with mild manganese toxicity precipitates violent behavior. These and other hypotheses certainly merit research in order to elucidate our findings, and provide direction for future cost-effective prevention and management of violence in our society.

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TABLE 1. SUBJECTS FOR THE THREE PHASES OF THIS STUDY.

PHASE	LOCATION OF PRISONERS	SUBJECTS	
		PRISONERS	CONTROLS
I	Deuel Vocational Institute, Stanislaus, CA	N=104 Male *39 Caucasians* *33 Hispanics* *32 Blacks* Mean Age=29	N=83, Male 52 Town Controls *42 Caucasians* *10 Hispanics* Mean Age=24 31 Guard Controls *20 Caucasians* *11 Blacks* Mean Age=31
II	Los Angeles & San Bernadino County Jails	N=60 Male *20 Caucasians* *20 Hispanics* *20 Blacks* Mean Age=27	N=42, Male 42 Town Controls *10 Caucasians* *10 Hispanics* *10 Blacks* Mean Age=22
III	San Bernadino County Jails	N=29 Male *29 Caucasians* Mean Age=27	N=59, Male 30 San Bernadino Town Controls *30 Caucasians* Mean Age=33 29 Orange County Town Controls *29 Caucasians* Mean Age=33

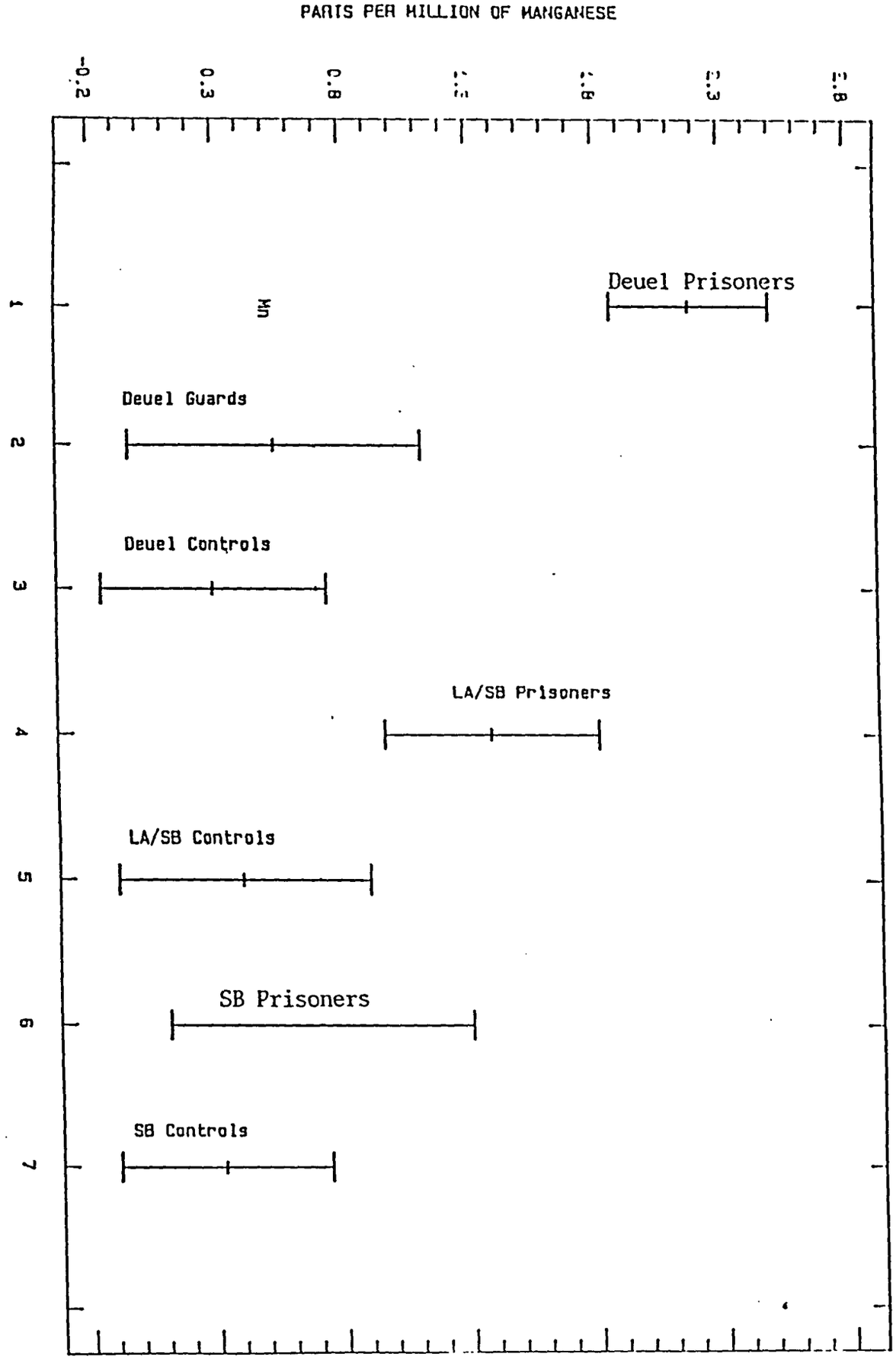

FIG 1

FIGURE 1.

HAIR MANGANESE LEVELS IN PRISON POPULATIONS AND CONTROLS